**Universidade de Aveiro** Departamento de Ciências Médicas
**Ano** 2018

**Raquel Catarina Saraiva Diaz**

# Methicillin resistant *Staphylococcus aureus*: *mec*C gene and vancomycin susceptibility

# *Staphylococcus aureus* meticilina resistente: gene *mec*C e susceptibilidade à vancomicina

**Universidade de Aveiro** Departamento de Ciências Médicas
**Ano 2018**

**Raquel Catarina Saraiva Diaz**

# Methicillin resistant *Staphylococcus aureus*: *mec*C gene and vancomycin susceptibility

# *Staphylococcus aureus* meticilina resistente: gene *mec*C e susceptibilidade à vancomicina

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciências e Tecnologias da Saúde, realizada sob a orientação científica do Doutor Bruno Gago, Professor Auxiliar Convidado do Departamento de Ciências Médicas da Universidade de Aveiro e co-orientação da Professora Doutora Vera Afreixo, Professora Auxiliar do Departamento de Matemática da Universidade de Aveiro.

Ao amor, à persistência e à dedicação.

**o júri**

Presidente

Professor Doutor João Manuel Nunes Torrão
Professor catedrático da Universidade de Aveiro

Doutor Nelson Fernando Pacheco da Rocha
Professor catedrático da Universidade de Aveiro

Doutor Samuel Martins Silvestre
Professor auxiliar da Universidade da Beira Interior

Doutora Nádia Isabel Almeida Osório
Professora adjunta do Instituto Politécnico de Coimbra

Doutora Bárbara Silva Rocha
Investigadora da Universidade de Coimbra

Doutor Bruno Miguel Alves Fernandes do Gago
Professor auxiliar convidado da Universidade de Aveiro

**agradecimentos**

O doutoramento era uma meta que sempre me propus a atingir. Foi um percurso longo e muito trabalhoso, um percurso nem sempre fácil de conciliar com a minha profissão, um percurso que me roubou muitas horas à minha vida pessoal mas um percurso, que hoje, me deixa feliz. Nada seria possível sem o apoio, motivação e orientação de todos os que se cruzaram comigo neste longo caminho.

Começo por agradecer aos Professores Doutores Luís Almeida e Nelson Rocha, por sempre nos motivarem e ajudarem em tudo o que estava ao seu alcance. Aos meus colegas de doutoramento, que estavam sempre disponíveis para uma palavra de motivação.

Ao Centro Hospitalar Baixo Vouga, nomeadamente ao Dr. Elmano Ramalheira, meu diretor de serviço, que sempre me apoiou e motivou neste percurso.

Ao meu orientador Professor Bruno Gago e co-orientadora Professora Vera Afreixo por tantas e tantas horas que me disponibilizaram. Foram incansáveis em todos os momentos, estiveram disponíveis sempre que precisei, foram sem duvida, o pilar de todo este trabalho. Não foram só orientadores, foram amigos. Obrigada!

À Carmen Rodrigues, um membro que fez parte da nossa equipa de investigação e com quem foi um prazer trabalhar. Obrigada por todas as horas e toda a dedicação colocadas neste projeto.

Aos meus amigos e à minha família, que são o melhor de mim. São os que tornam a minha vida mais fácil, mais simples e mais bonita. Não tenho qualquer duvida que tudo o que consigo é porque o amor é uma constante na minha vida. Tenho sempre alguém que me diz: É por aqui, é este o caminho e tu és capaz!

A todos...Obrigada!

**Palavras-chave**

Gene *mec*C, *Staphylococcus aureus*, Prevalência, *mec*CMRSA, métodos de rastreio, Vancomicina, "MIC creep", testes de susceptibilidade aos antibióticos.

**Resumo**

*Staphylococcus aureus* é uma das principais causas de infeção e a vancomicina o antibiótico de eleição para o seu tratamento. Em 2011 foi descoberto o gene *mec*C em isolados de *Staphylococcus aureus* resistentes à meticilina, um gene 69% semelhante ao gene *mec*A. Também recentemente foi descrito um fenómeno designado de "MIC creep", que traduz o aumento da concentração mínima inibitória da vancomicina dentro do intervalo de susceptibilidade. Os objectivos do presente trabalho incluíram avaliar a prevalência global do gene *mec*C em isolados de *Staphylococcus aureus* resistentes à meticilina e o fenómeno de "MIC creep" numa perspectiva global, recorrendo a técnicas de meta-analise. Em Portugal, a investigação deste fenómeno é ainda residual e como pode apresentar características regionais, este trabalho pretendeu também avaliar a sua presença em isolados de *Staphylococcus aureus* resistentes à meticilina no Centro Hospitalar Baixo Vouga, Aveiro.

Na avaliação da prevalência do gene *mec*C obtivemos uma prevalência global de 0.009 (95% CI = 0.005–0.013). Apesar da prevalência ser rara e, ausente em Portugal, é de extrema importância monitorizar a sua possível presença em isolados de *Staphylococcus aureus* resistentes à meticilina.

Na avaliação do fenómeno de "MIC creep", a nível global, os resultados obtidos não sugeriram a presença deste fenómeno. Os resultados mostraram um valor médio de concentração mínima inibitória da vancomicina de 1,19 mg/L e de 1,20 mg/L, determinados por Etest e microdiluição em caldo, respectivamente. A nível regional, os nossos resultados não mostraram evidência de "MIC creep" mas descobriram diferenças nas metodologias aplicadas para determinar o valor da concentração mínima inibitória da vancomicina.

Quer com a pesquisa do gene *mec*C, quer com a avaliação dos valores de concentração mínima inibitória da vancomicina, a finalidade do presente trabalho assenta na necessidade de compreender melhor a resistência das infeções hospitalares aos antibióticos numa das principais bactérias responsável por infeções nosocomiais e alertar atempadamente, quer os clínicos para a possibilidade de um cenário de resistência à vancomicina, quer o laboratório de microbiologia para a possibilidade da existência de estirpes portadoras do novo gene de resistência.

**Keywords**

*mec*C gene, *Staphylococcus aureus*, Prevalence, *mec*CMRSA, screening methods, vancomycin; MIC creep; susceptibility testing methods.

**Abstract**

*Staphylococcus aureus* is a major cause of infection and vancomycin the gold standard for its treatment. In 2011, *mec*C gene was discovered in methicillin-resistant *Staphylococcus aureus*, this gene shares 69% nucleotide homology with *mec*A gene. Also in recent years it has been described a phenomenon called "MIC creep", which describes the increase of the minimum inhibitory concentration of vancomycin within susceptibility range.

The aims of this work included the evaluation of the overall prevalence of *mecC* gene and the worldwide phenomenon of “MIC creep” using meta-analysis techniques. Since that in Portugal this investigation is still residual and because this is a phenomenon with regional characteristics, it was also assessed the presence of “MIC creep” in *Staphylococcus aureus* strains of Centro Hospitalar Baixo Vouga, Aveiro.

For the evaluation of *mecC* gene it was obtained an overall prevalence of 0.009 (95% CI = 0005-0013). Despite the rare prevalence of this gene and absence of suspected strains, it is of utmost importance in clinical and epidemiological terms to screen this new form of methicillin-resistant *Staphylococcus aureus*.

For the evaluation of global evidence of vancomycin “MIC creep”, our results do not suggest the presence of this phenomenon. The results obtained showed that mean values of vancomycin Minimum Inhibitory Concentration, of all methicillin-resistant *Staphylococcus aureus* isolates reported, were 1.19 mg/L and 1.20 mg/L determined by Etest and Broth Microdilution method, respectively. At the regional level, our results showed no evidence of “MIC creep” but described differences in methodologies applied to determine vancomycin Minimum Inhibitory Concentration.

Either with the evaluation of the Minimum Inhibitory Concentration of vancomycin values or with research of the *mec*C gene in *Staphylococcus aureus*, this work highlights the need to better understand the resistance of nosocomial infections to antibiotics and to alert clinicians to the possibility of resistance to vancomycin scenario and the microbiology laboratories for the risk of new strains carrying the resistance gene.

# Table of Contents

# Table of Figures



# Table of Tables

# Abreviations

| | |
|---|---|
| AST | Antimicrobial susceptibility testing |
| AUC/MIC | The ratio of the area under the serum drug concentration versus time curve and the MIC |
| BMD | Broth microdilution |
| BSI | Bloodstream infection |
| CA-MRSA | Community Acquired –Methicillin Resistant *Staphylococcus aureus* |
| CLSI | Clinical and Laboratory Standards Institute |
| Cmax | Maximum serum drug concentration |
| Cmax/MIC | Ration of serum concentration and the MIC |
| EARSS | European countries is the European Antimicrobial Surveillance System |
| ECDC | European Centre for Disease Prevention and Control |
| EUCAST | European Committee for Antimicrobial Susceptibility Testing |
| FDA | Food and Drug Administration |
| HA-MRSA | Hospital Acquired –Methicillin Resistant *Staphylococcus aureus* |
| HVR | Hypervariable Region |
| ISS | Integration Site Sequence |
| J region | Joining region |
| LA-MRSA | Livestock Acquired –Methicillin Resistant *Staphylococcus aureus* |
| *mec*CMRSA | *mec*C gene in Methicillin Resistant *Staphylococcus aureus* |
| MPD | Metallo Protease Domain |
| MRSA | Methicillin Resistant *Staphylococcus aureus* |
| MSCRAMMs | Microbial surface components recognizing adhesive matrix molecules |
| MSSA | Methicillin-Sensitive *Staphylococcus aureus* |
| MIC | Minimum Inhibitory Concentration |
| ORFs | Open Reading Frames |
| PBD | Penicillin Binding Domain |
| PBPs | Penicillin-Binding Proteins |
| PCR | Polimerase Chain Reaction |
| PVL | Panton - Valentine Leukocidin |
| *S.aureus* | *Staphylococcus aureus* |

| | |
|---|---|
| SCC*mec* | Staphylococcal Cassette Chromosome |
| SSTIs | Skin and Soft Tissue Infections |
| sRNA | Small RNAs |
| t | Time |
| t> MIC | The concentration of vancomycin remains above the MIC |
| VISA | Vancomycin Intermediate *Staphylococcus aureus* |
| hVISA | Heterogeneous VISA |
| VRE | Vancomycin resistant enterococci |
| VRSA | Vancomycin Resistant *Staphylococcus aureus* |
| VRSA | Vancomycin Resistant *S.aureus* |
| US | United States |

# Publications

The results presented in this thesis are part of the following list of publications, organized by category and descending chronological order:

## Papers

"Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections - a systematic review and meta-analysis". Clinical Microbiology and Infection. August 2017. PMID: 28648858. FI: 5,394

Evaluation of vancomycin MIC creep in *Staphylococcus aureus*". Journal of Global Antimicrobial Resistance. April 2017. PMID: 28751240. FI: 2,022

"Methicillin resistant *Staphylococcus aureus* carrying the new *mec*C gene – a meta-analysis." Diagnostic Microbiology and Infectious Diseases. December 2015. PMID: 26652130. FI: 2,341

## Posters:

"Meta-analysis of Vancomycin MIC creep in *Staphylococcus aureus* infections: sensitivity analysis of the method. 38th Annual Conference of the International Society for Clinical Biostatistics. Vigo, July 2017.

"Evaluation of vancomycin susceptibility among isolates of *Staphylococcus aureus* infections by broth microdilution and Vitek2, during 4 years in Aveiro, Portugal". European Congress of Microbiology and Infectious Diseases (ECCMID) 2016. Amsterdam, April 2016.

"Methicillin resistant *Staphylococcus aureus* carrying the new *mec*C gene – a meta-analysis". European Congress of Microbiology and Infectious Diseases (ECCMID) 2015. Copenhagen. April 2015.

## Oral session:

"Evaluation of vancomycin MIC creep in *Staphylococcus aureus* infections – a meta-analysis". European Congress of Microbiology and Infectious Diseases (ECCMID) 2017. Vienna. April 2017.

# Part I. Introduction

## 1 Motivation

Antimicrobial resistance is one of the great problems of clinical microbiology. Your detection is required for clinical antimicrobial susceptibility categorization, infection and public health purposes. Antimicrobial resistance of Gram-negative bacteria is widely studied, but less developed for Gram-positive bacteria. The rapid acquisition of antibiotic resistance by *Staphylococcus aureus* (*S.aureus*) is a significant problem for treatment of human infections caused by this organism. *S.aureus* is a highly successful opportunistic pathogen, a frequent colonizer of the skin and mucosa of humans and animals and can produce a wide variety of diseases. Actually, from all the known staphylococcal species, *S.aureus* is the most virulent. Regardless the big number of antistaphylococcal antibiotics that are available, its ability to adapt makes it one of the principal causes of morbidity or even mortality. This awareness, allied with the emergence of two new problems, the discovery of a new gene in *S.aureus* and a new phenomenon of MIC creep in vancomycin and the important role of clinical laboratory to monitoring these situations, are the main motivations of my research.

# 2 Problem definition

*S.aureus* is an important human pathogen and was first recognized as the etiological agent of suppurative abscesses more than 130 years ago (1). These infections range from mild skin and soft-tissue infections to life-threatening endocarditis, chronic osteomyelitis, pneumonia or bacteremia, which are associated with significant morbidity and mortality (2). The advent and use of antibiotics such as penicillin and methicillin in the mid 20th century initially proved to be effective against *S.aureus*. However, this microorganism rapidly acquired resistance to these antibiotics and infections with penicillin-resistant *S.aureus* and methicillin-resistant *S.aureus* (MRSA) appear and were difficult to treat. Although progress has been made, MRSA remains a significant threat to human health globally (1).

In the decade of 60s, when the resistance to methicillin was detected in *S.aureus*, the glycopeptide antibiotics where selected as the gold standard to threat some severe infections of MRSA. Vancomycin was especially used (3, 4). A phenomenon named "MIC creep" was described in recent years denoting a slow but steady increase in vancomycin Minimum Inhibitory Concentration (MIC) observed over time (4, 5). Poor clinical outcomes, like delayed response, bigger rate of replace, extended duration of hospital admission or even mortality are being associated to patients with isolates that reveal MIC creep (6-8). The visible growth in vancomycin MIC in MRSA isolates that was noticed in recent years can anticipate the appearance of isolates entirely resistant. The pool of publications related to MIC creep showed some inconsistencies about this phenomenon and is important understand this phenomenon as a regional problem, as a global problem or as a problem that don't exist because treatment response can be influenced by increased in vancomycin MIC value.

At the side of this problem it was discovery a new *mec*A gene homolog, *mec*C gene, that was found in isolates from both humans and animals. This gene shares 69% nucleotide homology with *mec*A, the gene responsible for resistance to methicillin (9). The detection, antimicrobial susceptibility testing, and treatment for *mec*CMRSA are not different from other MRSA strains. However, molecular

techniques such as detection of MRSA with Polimerase Chain Reaction (PCR) or slide agglutination tests do not detect *mec*CMRSA. The use of these techniques, for detection or for confirmation of MRSA, can lead to false-negative results, and *S.aureus* will be incorrectly diagnosed as methicillin susceptible. There are some public health concerns about this new gene (*mec*C) due to the fact that either the tests, phenotypic or genotypic, are not sufficient to detect this gene (10).

With the discovery of the *mec*C gene in *S.aureus* and the description of the phenomenon "vancomycin MIC creep" the treatment response in case of the infections with this microorganism could be compromised.

# 3 State of the art

## 3.1 *Staphylococcus aureus*

Alexander Ogston, in 1882, described Staphylococci and classified as *Staphylococcus* (from the Greek words staphylos ["grape"] and kokkos ["berry" or "seed"]) (11). Two years later, Friedrich J. Rosenbach, reported 2 pigmented colonies of staphylococci and suggest the nomenclature *Staphylococcus albus* (Latin for "white") and *S.aureus* (from the Latin aurum ["gold"]) (12).

Nowadays, from all the known staphylococcal species, *S.aureus* is the most virulent. Its great versatility makes this microorganism a big cause of mortality and morbidity despite all the effective antibiotics that are available (2). *S.aureus* is a pluripotent pathogen and toxin-mediated and non–toxin-mediated mechanisms are used by it to cause disease. This organism creates a large range of infections, nosocomial and community-based that can go from a simple skin and soft tissue infections (SSTIs) to systemic infections (13).

*S.aureus* is present on the population and constitutes the normal microbial flora so big number of healthy persons (25-50%) are colonized with it continuously or during a period of time (14). The biggest rates of colonization are amongst patients with insulin-dependent diabetes, HIV-infected, individuals submitted to haemodialysis and patients with some kind of skin damage. The colonization is more usual on the anterior nares but other parts can also be colonized like the skin, axilla, vagina, oropharynx and perineum. All of these colonization sites become a deposit of strains for future infections (2, 15). It is responsible for a high number of community-based infections of the skin, soft tissues and respiratory or infective endocarditis (15). Home infusion therapy, that keeps growing nowadays, is also responsible for some cases of community-acquired infections (2).

Overall, *S.aureus* is responsible for the majority of surgical wound infections and has one of the biggest percentages of all the nosocomial infections (16). Some individuals that are infected with *S.aureus* obtained the strains from environmental exposures or other people but the most common cause is with

their own colonisations (15). Transmission is very frequently due to the hands of hospital personnel, which transfer strains between patients (13).

### 3.1.1 Pathogenesis of Infection

In the pathogenesis of *S.aureus* infections there are five different stages: colonization, local infection, systemic dissemination and/or sepsis, metastatic infection and toxinosis (17). This microorganism is highly pathogenic due its virulence factors, structural and secreted products, playing a role in the pathogenesis of infection.

### 3.1.2 Regulation and Virulence determinants

*S.aureus* is equipped with regulatory systems that sense environmental conditions and respond by fine-tuning the expression of given metabolic and virulence determinants (17, 18).

At least three families of regulatory elements interlink to adjust gene expression to the specific environmental conditions: first, two component regulatory systems, of which *agr* is a paradigm; second, DNA-bindings proteins, largely represented by the Sar family of proteins and third, small regulatory RNAs (19).

*agr* functions as a quorum sensing control that reacts to bacterial density, allowing the preferential expression of surface adhesins during the exponential phase of growth and switching to the expression of exoproteins during the post exponential and stationary growth phases (20, 21).

Sar stands for staphylococcal accessory regulator. It is important locus that encodes a DNA-binding protein, SarA, positively controls *agr* and directly regulates adhesion genes (22).

Small RNAs (sRNA) are increasingly recognized as major players in regulation of gene expression (19).

### 3.1.3 Cell surface determinants involved in pathogenesis

*S.aureus* is extremely well equipped in surface factors and secreted proteins that mediate host colonization and disease (table I).

Biofilm is an extracellular polysaccharidic and proteinaceous network that gathers bacterial communities within a mechanically cohesive scaffold. Biofilm trapped bacteria are inactive and thus phenotypically tolerant to antibiotic induced killing. His formation is a major therapeutic problem. Biofilm production was mainly described in *S.aureus* in the settings of colonization of catheters and biomaterials (19, 23).

The majority of clinical *S.aureus* strains (>90%) elaborate a polysaccharidic capsule. Capsule type 5 and type 8 are responsible for up to 75% of clinical infections, they are both antiphagocytic and can increase virulence in several animal models (19).

Surface adhesins, confer adherence to a variety of host matrix proteins. These microbial surface components reacting with adherence matrix molecules are reassemble under the acronym MSCRAMM "Microbial Surface Components Recognizing Adhesive Matrix Molecules". Most are covalently bound to the cell wall peptidoglycan (24). Relevant MSCRAMMs for pathogenesis include clumping factor B for colonization of nasal epithelia, clumping factor A and fibronectin-binding proteins A and B for endocarditis, collagen-binding protein for osteomyelitis and protein A for immune escape and promotion of experimental osteoarthritis (18).

Teichoic and lipoteichoic acids, represents up to 50% of the dry weight of purified staphylococcal walls. They have a physiologic role of great importance in cell wall metabolism and are probably the site of attachment of cell active enzymes and others proteins (19, 25).

Peptidoglycan is a highly conserved constituent of the Gram-positive envelope. Is a major scaffold for anchoring most MSCRAMMs. It plays a role in pathogenesis (19).

### 3.1.4 Secreted enzymes and hemolysins

*S.aureus* produces a number of exoenzymes, membrane-active proteins (hemolysins and leucocidins) and toxins that are involved in disease mechanism. Exoenzymes encompass proteases and lipases, witch are destructive regarding host tissues and useful for getting nutrient to the invading bacterium (19). *S.aureus* has a minimum of four hemolysins referred to as α-hemolysin, β-hemolysin, γ-hemolysin, δ- hemolysin. They can lyse erythrocytes and other eukaryotic cells. They are all encoded on the chromosome and are subject to *agr* regulation (19). Panton-Valentine Leukocidin (PVL), unlike the other hemolysins, is encoded by a mobile phage that can transfer PVL to others strains. The prevalence rate of PVL is usually low (≤ 2%) in MSSA and health care-associated MRSA (26), whereas it is present in almost 100% of isolates of CA-MRSA (27, 28). PVL-producing *S.aureus* is associated with skin and soft tissue infection and severe haemorrhagic pneumonia in children and young adults (18).

**Table I: Virulence factors of *Staphylococcus aureus* and their proposed pathogenic mechanisms. Adapted (18)**

| | |
|---|---|
| Involved in evading/destroying host defenses | Microcapsule Protein A Coagulase<br>Fatty acid–metabolizing enzyme<br>Leukocidin and/or g-toxin |
| Involved in tissue invasion/penetration | Proteases<br>Nucleases<br>Lipases<br>Hyaluronate lyase Staphylokinase |
| Involved in toxin-mediated disease and/or sepsis | Toxic shock syndrome toxin<br>Enterotoxins<br>Cytolytic toxins (a, b, g, and d) |
| Induce specific toxinosis | Toxic shock syndrome toxin<br>Enterotoxin<br>Exfoliative toxin |
| Attach to endothelial cells and basement membrane | Binding proteins for fibrinogen, fibronectin, laminin, collagen, |

### 3.1.5 Clinical Manifestations

*S.aureus* is responsible for local infections or acts at a distance by secretion of toxins (table II) (19). The SENTRY antimicrobial surveillance program that collects data from United States (US), Europe, Canada, Latin America and the Western Pacific, reported the following distributions of *S.aureus* infections: 39,2% of SSTI; 23,2% of lower respiratory tract infections; 22% of bloodstream infections, including infective endocarditis and 15,6% of other infections, including infections of the urinary tract, brain and abdominal cavity (29).

*S.aureus* SSTIs include primary pyoderma (folliculitis, furuncles, carbuncles and impetigo) and soft tissue infections (cellulitis, erysipelas and pyomyositis). They are commonly classified according to the anatomic structure involved: infection of the epidermis (impetigo); infection of the superficial dermis (folliculitis); infection of deep dermis (furuncles, carbuncles and hidradenitis suppurative) and infection of subcutaneous cellular tissues (cellulitis, fasciitis, erysipelas and pyomyositis) (2). The diagnosis of SSTIs is usually made clinically. The basic anatomic lesion induced by *S.aureus* is a pyogenic exudate or an abscess. The more severe infections are usually associated with deeper tissue invasion. Superficial infections can often be treated with local care, surgical drainage and, rarely, systemic antibiotics. On the other hand, deeper infections such as erysipelas, lymphangitis, lymphadenitis, cellulitis and necroting fasciitis are severe diseases that may be life threatening. They require hospitalization, systemic antibiotic therapy and prompt surgical drainage (13, 19).

Bloodstream infection (BSI) is defined as one or several positive blood cultures associated with general symptoms such as fever or hypotension (13). *S.aureus* is the second most common cause of BSI, with an overall contemporary incidence rate between 20 and 30 episodes per 100,000 inhabitants per year across the world (30). BSI is usually divided in two categories: nosocomial BSI, in which positive blood cultures occur two days or more after hospital entry and community acquired BSI, which occurs in the community or before two days of hospitalization (19). These are commonly related with the presence of intravascular catheters or devices, procedures in contaminated sites, surgical site infection and, sometimes, *S.aureus* pneumonia (19). A therapy with

antibiotics should be started if *S.aureus* is detected in any blood culture. Additionally, blood should be sampled for follow-up cultures and determination of the origin and dimension of the infection (31). An important consideration in the bacteraemia is the risk of endocarditis. The infective endocarditis is uniformly lethal if not treated with antibiotics, with or without surgery. This disease typically follows an acute course, with multiple peripheral septic emboli, valve destruction, myocarditis and mixed cardiogenic and septic shock (19).

In the hospital, *S.aureus* is becoming the most frequent pathogen responsible for nosocomial pneumonia. In community, pneumonia occurs primarily in elderly patients (> 75 years) admitted from nursing homes but also in patients with predisposing factors, such as diabetes and alcoholism (13, 19).

*S.aureus* is the most common pathogen in all three major classes of osteoarticular infection, namely, osteomyelitis, native joint septic arthritis and prosthetic joint infection (13).

**Table II: Common illnesses caused by *Staphylococcus aureus.* Adapted (2).**

| | |
|---|---|
| Skin and Soft Tissue Infections | Folliculitis<br>Furuncle, carbuncle Cellulitis<br>Impetigo<br>Mastitis<br>Surgical wound infections |
| Musculoskeletal Infections | Septic arthritis<br>Osteomyelitis<br>Pyomyositis<br>Psoas abscess |
| Respiratory Tract Infections | Ventilator-associated or nosocomial pneumonia<br>Septic pulmonary emboli<br>Postviral pneumonia<br>Empyema |
| Bacteremia and Its Complications | Sepsis<br>Septic shock<br>Metastatic foci of infection (kidney, joints, bone, lung)<br>Infective endocarditis |
| Infective Endocarditis | Injection drug use associated<br>Native-valve<br>Prosthetic-valve<br>Nosocomial |
| Toxin-Mediated Illnesses | Toxic shock syndrome<br>Food poisoning<br>Staphylococcal scalded-skin syndrome |

## 3.2 Methicillin Resistant *Staphylococcus aureus*

In the beginning of the 40s, the launch of penicillin G improved significantly the prognosis but in 1942 resistant strains started to emerge (32). A penicillinase enzyme that hydrolyzed the β-lactam ring and inactivated the drug was their resistance mechanism. This enzyme is encoded by blaZ, which typically resides on a large transposon on a plasmid. The rate of resistance to penicillin is now greater than 90% in human *S.aureus* isolates, making the use of penicillin essentially useless to treat these infections (33).

In order to stop the spread of this resistance to penicillin a semisynthetic penicillinase-resistant β-lactam that was named Methicillin was created. This new antibiotic was launch in 1959 with the commercial name "Celbenin" but the earliest reports of resistant strains started to appear just after the next year in London (34). The isolates had the same phage type and included isolates taken on the same day from a patient and a nurse on the same ward. This discovery advertised to the emergence of methicillin-resistant *S.aureus* (MRSA) as a nosocomial pathogen. MRSA emerged during the 1960s in many countries, even tin countries where methicillin was not available, and it is now very frequent worldwide (35).

Despite that Methicillin is out of the market and the clinic, the term MRSA continued to exist. It is used to identify the resistance to all β-lactams (except the last generation of cephalosporins). The infection's treatment can become even more complicated because MRSA can become resistant to one or more different antimicrobials (36). This resistance includes vancomycin, considered one of the last treatment options for severe MRSA infections, and other relatively new agents like linezolid and daptomycin (33, 37).

### 3.2.1 Mobile resistance element SCC*mec*

Microorganisms are capable of spreading, creating ecological reservoirs, colonizing and causing diseases.

The mobile genetic elements that are involved in the resistance and virulence in staphylococci are bacteriophages, chromosomal cassettes, plasmids, insertion sequences and transposons (38).

MRSA contains one resistance island called *staphylococcal cassette chromosome* (SCC*mec*), where is the genetic element that confers resistance to methicillin. SCC*mec* is an exogenous piece of DNA that may vary between 15 and 60kb (19) and your basic elements are the ccr gene complex, the *mec* gene complex and the joining region (J region) (38, 39) (figure 1). Is located near the replication origin of the chromosome of *Staphylococcus* and inserted at the insertion site *att*B (located at the 3' end of *orf*X). It is a mobile genetic element carrying the *mec* gene (*mec*A, *mec*B and *mec*C) along with the genes that control its expression, *mec*R1 (encoding the signal transduce protein MecR1) and *mec*I (encoding the repressor protein *Mec*I), and acts as a carrier to exchange genetic information between *Staphylococcus* strains (9, 40, 41).

The *ccr* gene complex is composed by *ccr* gene(s) and surrounding open reading frames (ORFs) several of them without any known function. Three phylogenetically distinct *ccr* genes, *ccr*A, *ccr*B and *ccr*C, have been identified in *S.aureus* (38, 42). Additionally, SCC*mec* is integrated to the chromosome of *Staphylococcus* strains by the accurate excision and integration of *ccr*AB or/and *ccr*C. According to the different kinds of *ccr*AB or/and *ccr*C, the *ccr* gene complex was classified into 8 allotypes: type 1 carrying *ccr*A1 and *ccr*B1 genes, type 2 carrying *ccr*A2 and *ccr*B2 genes, type 3 carrying *ccr*A3 and *ccr*B3 genes, type 4 carrying *ccr*A4 and *ccr*B4 genes, type 5 carrying *ccr*C1 gene, type 6 carrying *ccr*A5 and *ccr*B3 genes, type 7 carrying *ccr*A1 and *ccr*B6 genes, and type 8 carrying *ccr*A1 and *ccr*B3 genes (43).

The *mec* gene complex is constituted of *mec*A, *mec*B and *mec*C, its regulatory genes (*mec*R1 and *mec*I) and associated insertion sequences. Is classified into 5 classes take into account the regulatory genes located upstream and downstream of the *mec* gene and the difference of insertion sequences (43). The class A *mec* gene complex is the prototype complex, which contains *mec*A, the complete *mec*R1 and *mec*I regulatory genes upstream of *mec*A, the hyper-variable region (HVR) and insertion sequence IS431 downstream of *mec*A. The class B *mec* gene complex is composed of *mec*A, a truncated *mec*R1 resulting

from the insertion of IS1272 upstream of *mec*A, HVR and IS431 downstream of *mec*A. The class C *mec* gene complex contains *mec*A, truncated *mec*R1 by the insertion of IS431 upstream of *mec*A, HVR and IS431 downstream of *mec*A. There are two distinct class C *mec* gene complexes; in the class C1 *mec* gene complex, the IS431 upstream of *mec*A has the same orientation as the IS431downstream of *mec*A (next to HVR), while in the class C2 *mec* gene complex, the orientation of IS431 upstream of *mec*A is reversed. The class D *mec* gene complex is composed of *mec*A and Δ*mec*R1, but does not carry an insertion sequence downstream of Δ*mec*R1 (42, 44).

Apart from *ccr* and *mec,* SCC*mec* has some not essential components called J-region (Junkyard-region). Differences in the J regions leads to some variants of SCC*mec* types (38, 45). On the basis of location in *SCC*mec, the J region was classified into J1, J2 and J3 regions (43).

Currently, based on the nature of *mec* and *ccr* genes complexes and different subtypes in accordance with their J region DNA segments, eleven types of SCCmec (type I to XI) are known (table III) (46).

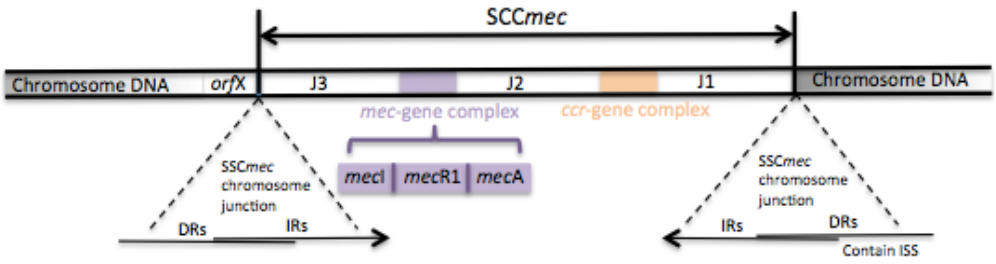


**Figure 1: Basic structure of SCC*mec*.**
SCC*mec* is bracketed by direct repeats (DRs) that contain integration site sequence (ISS). A pair of inverted repeats (IRs) is present at the termini of SCCmec. Two critical gene complexes, *ccr* and *mec* are present, and the other regions are designated J1, J2, and J3. Adapted (40).

**Table III: Current SCC*mec* types. Adapted (43)**

| SCC*mec* type | *mec* gene complex | Structure of *mec* gene complex | *ccr* gene complex | *ccr* genes |
|---|---|---|---|---|
| **I** | Class B | IS1272-Δ*mec*R1-*mec*A-IS431 | Type 1 | *ccr*A1, *ccr*B1 |
| **II** | Class A | *mec*I-*mec*R1-*mec*A-IS431 | Type 2 | *ccr*A2, *ccr*B2 |
| **III** | Class A | *mec*I-*mec*R1-*mec*A-IS431 | Type 3 | *ccr*A3, *ccr*B3 |
| **IV** | Class B | IS1272-Δ*mec*R1-*mec*A-IS431 | Type 2 | *ccr*A2, *ccr*B2 |
| **V** | Class C2 | IS1431-*mec*A- Δ*mec*R1-IS431 | Type 5 | *ccr*C1 |
| **VI** | Class B | IS1272-Δ*mec*R1-*mec*A-IS431 | Type 4 | *ccr*A4, *ccr*B4 |
| **VII** | Class C1 | IS1431-*mec*A-Δ*mec*R1-IS431 | Type 5 | *ccr*C1 |
| **VIII** | Class A | *mec*I-*mec*R1-*mec*A-IS431 | Type 4 | *ccr*A4, *ccr*B4 |
| **IX** | Class C2 | IS1431-*mec*A- Δ*mec*R1-IS431 | Type 1 | *ccr*A1, *ccr*B1 |
| **X** | Class C1 | IS1431-*mec*A- Δ*mec*R1-IS431 | Type 7 | *ccr*A1, *ccr*B6 |
| **XI** | Class E | blaZ-*mec*A-*mec*R1-*mec*I | Type 8 | *ccr*A1, *ccr*B3 |

### 3.2.2 Mechanism of resistance in Methicillin Resistant *Staphylococcus aureus*

Peptidoglycan is the main structural component of the cell wall, and it consists of glycan strands made of repeating N-acetylglucosamine and N-acetylmuramic acid disaccharides connected by peptide cross-links between N-acetylmuramic acid moieties on adjacent strands (33).

In summary, the steps in cell wall biosynthesis in staphylococci are N-acetylglucosamine and N-acetylmuramic acid disaccharides attached via a β-1,4-glycosidic bond to the reducing end of the growing peptidoglycan chain in a transglycosylation reaction. The recently included repeating unit is cross-linked by a transpeptidation reaction to a stem peptide in an adjacent peptidoglycan strand. Both transglycosylation and transpeptidation are carry out by penicillin-binding proteins (PBPs). The stem peptide composition varies between bacterial species, but is typically L-Ala–γ-D-Glu–L-Lys–D-Ala–D-Ala in gram-positive bacteria (47).

Differently from the penicillin resistance of *S.aureus*, methicillin resistance is not mediated by a plasmid-borne β-lactamase and it was described in early studies as intrinsic resistance (33). Other bacterial pathogens with identical intrinsic

resistance to β-lactams are related to changes in PBPs, either in their amount or in their affinity for β-lactams, so attention turned to PBPs in MRSA as the likely mechanism (33).

*S.aureus* generally produces four PBPs. These enzymes are bounded to cytoplasmic membrane and their functions are the assembly and regulation of the latter stages of the cell wall biosynthesis (48). These enzymes are susceptible to modification by β-lactam antibiotics leading to the bacterial cell wall biosynthesis inhibition and bacterial death. PBP2a is refractory to the action of β-lactam antibiotics because is capable of assuming the functions of the four typical PBPs in the presence of β-lactam antibiotics (41). In brief, MRSA is resistant to almost all β-lactam antibiotics. In methicillin-sensitive *S.aureus* (MSSA), the β-lactam antibiotics bind to the native PBPs that are present in the *S.aureus* cell wall, which results in the disruption of the synthesis of the peptidoglycan layer and the *S.aureus* will not survive (48). On the other hand, in MRSA the β-lactam antibiotics are not able to bind due to the presence of PBP2a, leading to the increase of MRSA because there is no disruption of peptidoglycan layer synthesis (49, 50).

PBP2A is encoded by an acquired gene *mec*A gene located on the chromosome of MRSA (41) and SCC*mec* carry this gene and was responsible for the widely disseminated among staphylococcal species (51).

*mec*A is regulated by the repressor MecI and the transmembrane β-lactam-sensing signal-transducer MecR1, which are divergently transcribed. MecI represses the transcriptions of *mec*A and *mec*R1–*mec*I if a β-lactam antibiotic is absent. However, with antibiotic, *mec*R1 is autocatalytically cleaved and the metalloprotease domain (located in the cytoplasmic part of *mec*R1) becomes active. This metalloprotease, which is bounded to the *mec*A gene, cleaves *mec*I enabling the transcription of *mec*A and the production of PBP2a (figure 2) (38, 50, 51).

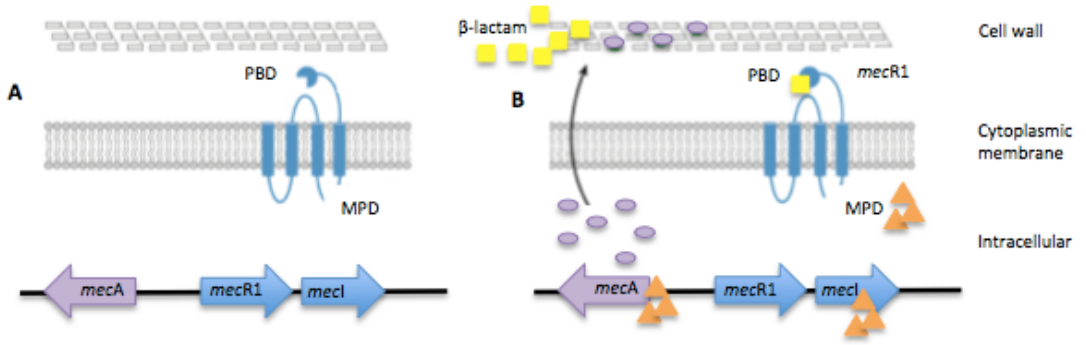


**Figure 2: Model of the salient features of *mec*A regulation.**
**(A)** Absence of β-lactams: the binding of the repressor MecI to this region stops transcription from the *mec* operator. **(B)** Presence of β-Lactams: are detected by their binding to the penicillin-binding domain (PBD) of MecR1. MecR1 is autocatalytically cleaved and the metalloprotease domain (MPD) becomes active. This MPD, which is bound to the *mec*A, cleaves *mec*I allowing the transcription of *mec*A and the production of PBP2a. Adapted (33).

### 3.2.3 Epidemiology of Methicillin Resistant *Staphylococcus aureus*

Surveillance of MRSA gives relevant information on the extent of the MRSA epidemic, identifying priorities for infection control and the need for any adjustment in antimicrobial drug policy and guides intervention programs. In Europe, there is only one initiative that continuously monitors antimicrobial resistance, the European Antimicrobial Surveillance System (EARSS). This international network connects national surveillance systems and provides results of routine antimicrobial susceptibility tests (AST) following standardized protocols that can be comparable and validated between a representative set of laboratories of multiple countries (52).

From the start of the year 1999 to the end of 2002, EARSS received AST results of 53,264 *S.aureus* blood isolates from 27 different countries. 20% of the total isolates were identified as methicillin resistant. MRSA was more frequently isolated from men (21%) than from women (18%, $p < 0.001$). In average patients with a blood culture positive for MRSA were older than patients with MSSA (mean age, 65.3 [SD 18.7] versus 58.6 [SD 23.4], $p < 0.001$). Patients admitted to intensive care units have the highest proportion of MRSA (35%) (53).

Geographically there is an obvious difference between the north and the south of Europe. The countries in the northern Europe have a lower MRSA prevalence when compared with the southern countries (54).

Before the appearance of antibiotics, *S.aureus* bacteremia was fatal in most of cases. In a study of cases in the beginning of 1940s, the mortality rate on 122 consecutive patients was 82% and 98% in patients older than 50 years.

Nowadays, *S.aureus* is carried on the skin or mucous membranes of approximately 25 to 35% of healthy humans (55). The MRSA and penicillin-resistant *S.aureus* evolution is very similar (56). MRSA is currently pandemic, with dissemination of Hospital Acquired – MRSA (HA-MRSA) clones from the 1960s, Community Acquired – MRSA (CA-MRSA) clones from the 1990s and Livestock Acquired – MRSA (LA-MRSA) clones from the 2000s (55).

In some European countries, such as, Greece, Austria, Ireland, France and the UK the prevalence of HA-MRSA decreased in the last years. The MRSA clones most frequently collected from all continents from 1961 to 2008 were CC5, CC8, CC22, CC30 and CC45 (35, 57). The most common in Europe and USA is CC45 (55).

In the last decades, MRSA infections were principally confined to hospitals, but the emergence of CA-MRSA infections shaped the epidemiology of *S.aureus* leading to the replacement of HA-MRSA clones by community-associated MRSA (58). Between 2009-2010 Tavares A, *et al*. studied a total of 1,487 *S.aureus* isolates collected from 16 Portuguese healthcare institutions located in different geographic regions of Portugal, reporting that MRSA clones present in the community were clones typically found in hospitals (10, 51).

The study of Rolo, J., *et al* aimed to describe the population structure of CA-*S.aureus* in European countries, including some of the most populous countries in Europe like The Czech Republic, Spain, The Netherlands, Greece, United Kingdom, Sweden, Hungary, Bulgaria, Denmark, France, Poland, Romania, Portugal, Finland, Slovakia and Italy. The combination of the results obtained showed that 59% of isolates were related to epidemic CA-*S.aureus* clones, while 27% were related to HA clones. In CA-*S.aureus* epidemic clones, 58% were MRSA, 37% were related to USA300 and 36% were related to the European epidemic clones (51).

Based on the 2015 ECDC (European Centre for Disease Prevention and Control)

report, Portugal is the third European country with the highest prevalence of MRSA (46,8%), nevertheless is one of the countries that prevalence remains stable in the last years (Figure 3). The MRSA isolates were more frequent patients older than 65 years old (54).

Taking into account the prevalence of MRSA in European countries, the prevalence in Portuguese hospitals has been close to 50% for more than 10 years and remains one of the highest in Europe (Figure 4) (54), so is extremely important to our country to design national intervention and prevention strategies. It is also important to monitor changes in epidemiology of *S.aureus* infections (humans and animals) in order to provide the correct treatment, control the infection effectively and study the evolution of the species (55).

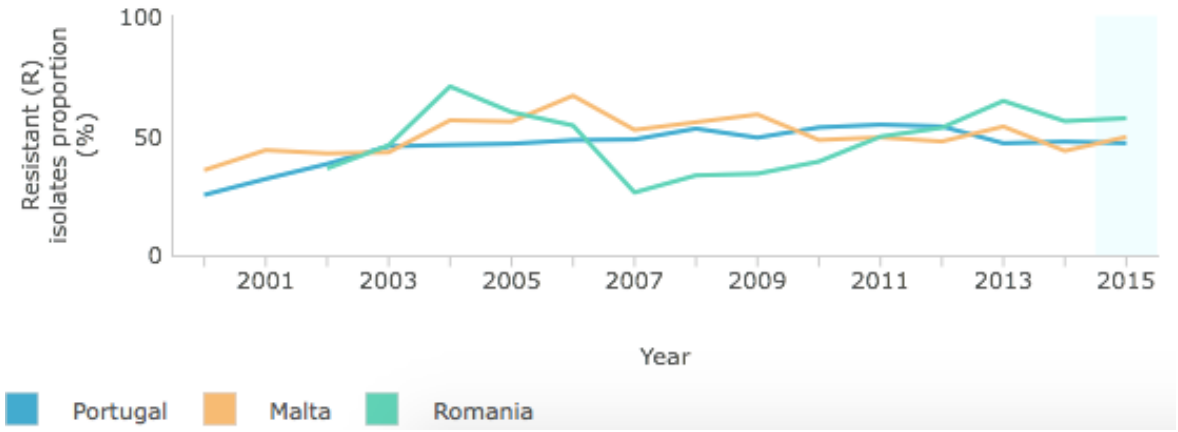


**Figure 3: Evolution of MRSA in the three countries with highest prevalence. Adapted (54).**

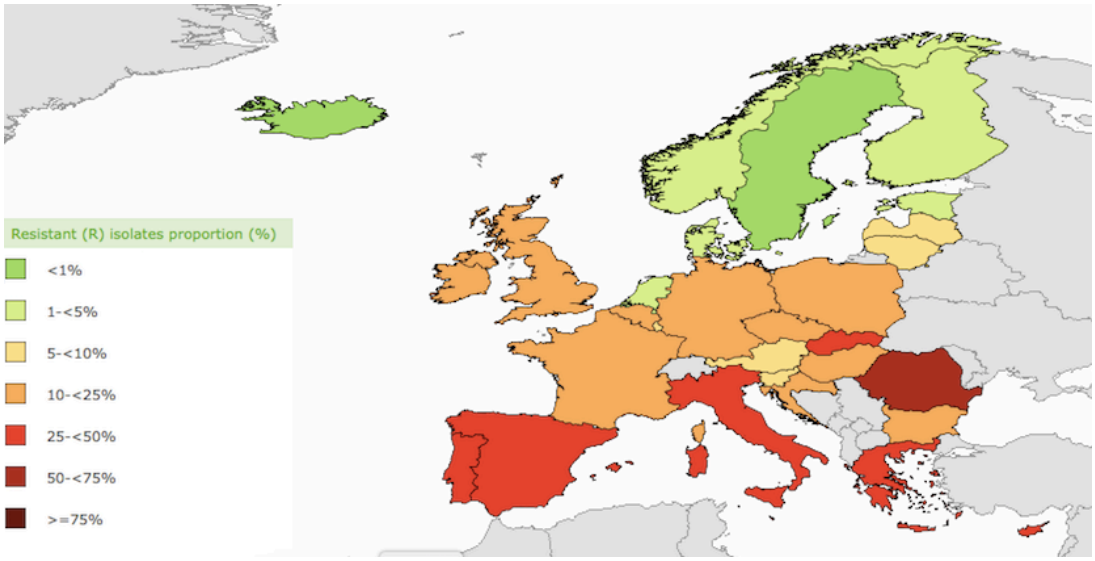


**Figure 4: Proportion of Methicillin Resistant *Staphylococcus aureus* isolates in participating countries in 2015. Adapted (54).**

### 3.2.4 Diagnostic detection of Methicillin Resistant *Staphylococcus aureus*

In a clinical microbiology laboratory, the correct detection and diagnosis of MRSA is extremely important to select the correct treatment and for infection control. The molecular identification of *mec*A gene, normally using PCR, or PBP2a, typically using antibody detection with slide agglutination assays are the recommended methodologies for MRSA detection. These methodologies are consistent because both *mec*A and PBP2a have a high level of conservation between MRSA isolates (10, 59).

Disk diffusion method consistently predicts methicillin resistance thought the cefoxitin screening. The MRSA isolates are resistant to cefoxitin when MIC is greater than 4mg/L (60).

### 3.2.5 The emergence of *mec*C Methicillin Resistant *Staphylococcus aureus*

In 2007, was isolated a *S.aureus* $LGA_{251}$ from bovine mastitis in England which was phenotypically MRSA but methodologies applied to detect *mec*A gene and

PBP2a were negative (61). Genome sequencing of this isolate found that the strain carried a new *mec*A homologue (*mec*$A_{LGA251}$) is only 69% identical to the conventional *mec*A at the DNA level and the encoded PBP2a was only 63% identical at the amino acid level. These facts explain the resistance described and the negative results obtained in the two methodologies applied (10, 62).

*mec*$A_{LGA251}$ is located within a SSC*mec* element inserted into the 3' region of orfX like the traditional *mec*A (10). In the end of 2009, SCC*mec* sequence from LGA251 was classified and given the designation of type XI SCC*mec.* In 2012 the gene *mec*$A_{LGA251}$ was renamed to *mec*C (9). The designation *mec*B (divergent homologue of *mec*A) was already described in *Macrococcus caseolyticus* (9, 10).

There are significative distinctions in the performance of the proteins encoded by *mec*C and *mec*A in methicillin resistance. The protein PBP2a$_{mecC}$ was bound by β-lactams but indicated greater affinity for oxacillin when compared with cefoxitin, while PBP2a$_{mecA}$ showed less affinity (63).

### 3.2.6 Diagnostic detection of *mec*C Methicillin Resistant *Staphylococcus aureus*

*mec*CMRSA have a high probability of being correctly identified as MRSA with antimicrobial susceptibility testing (64). Using automated system Vitek2, *mec*CMRSA produce a different profile when compared with *mec*AMRSA. When the antibiotics oxacillin and cefoxitin are tested, *mec*AMRSA is classically resistant to both and *mec*CMRSA is resistant to cefoxitin but susceptible to oxacillin, nevertheless they are reported as MRSA (65).

In clinical laboratories, the main problem is when the identification or confirmation of MRSA is done using molecular detection of the *mec*A gene. Using this method needs to consider the incorporation of *mec* gene primers that are able to amplify both *mec*A and *mec*C or the addition of *mec*C-specific primers. Also, slide agglutination assays for *mec*A encoded PBP2a will fail when identifying *mec*CMRSA. Using only the slide agglutination assays produce false negative results and is essential the confirmation by PCR (10). In brief, clinical microbiology laboratories must know that there is a potential diagnostic gap and

different tests should be used to correctly and rapidly identify *mec*CMRSA as MRSA.

### 3.2.7 Epidemiology of *mec*C Methicillin Resistant *Staphylococcus aureus*

Multiple theories regarding the origin, epidemiology and the impact of the MRSA isolates carrying the *mec*C gene were created since its discovery. Several studies were implemented aiming to understand this new gene present in *S.aureus* strains. The discovery of *mec*CMRSA in dairy cows in England, described by Garcia Alvarez, *et al*., indicates that these animals might provide a reservoir of infection (62).

The few available reports on this topic show that data on *mec*CMRSA in humans and animals are now becoming available, allowing some important information on the epidemiology and prevalence of *mec*CMRSA. At the time of this analysis, *mec*CMRSA was reported in 14 European countries and has been isolated from 14 host species. MRSA harbouring *mec*C has been reported from humans, livestock, domestic and wild animals (10, 66). The multilocus sequence types CC130 and ST425 are the major lineages found in *mec*CMRSA isolates (10).

## 3.3 Vancomycin

The vancomycin was discovery by Eli Lilly during the 50s. Since its discovery, more than 50 years ago, the interest in vancomycin keeps increasing. Studies published in the last years showed that there is more knowledge regarding this antibiotic and its use (3). Vancomycin became the gold standard in treatment of serious MRSA infections after the emergence of these strains (3, 4). Lilly isolated an organism called *Amycolatopsis orientalis* (previously known as *Streptomyces orientalis* and *Nocardia orientalis*) from a sample of dirt sent by a missionary that

visited the Borneo Island in Asia. This organism produced a substance that inhibited gram-positive organisms (compound 05865). Its brown colour gave it the name "Mississippi mud". The first clinical trials started in the middle of 1950s and the U.S. Food and Drug Administration (FDA) approved it for use in 1958 (3, 67).

### 3.3.1 Mechanism of Vancomycin Action

In Gram-positive bacteria, vancomycin inhibits the cell wall synthesis with a different mechanism than β-lactam antibiotics (68). While these antibiotics inhibit cell wall synthesis by binding to the transpeptidase active site of penicillin binding proteins, vancomycin binds to the C-terminal D-Ala-D-Ala residue of the peptidoglycan precursor and forms a stable, noncovalent complex, which prevents the use of the precursor for cell wall synthesis (Figure 5) (67, 69). To inhibits late-stage of peptidoglycan biosynthesis, vancomycin acts outside of cytoplasmatic membrane (68).

The vancomycin complex involves hydrogen bonds between the peptide component of vancomycin and the D-Ala-D-Ala residue. If any process interferes with the binding of the drug with D-Ala-D-Ala residues in the cell wall, the effectiveness of the vancomycin decrease (67). In *S. aureus*, the main location for cell wall synthesis is the division septum, so vancomycin has to diffuse to the tip of the division septum to bind to peptidoglycan precursors. This location and the distance of this diffusion depend on the cell cycle (67, 69).

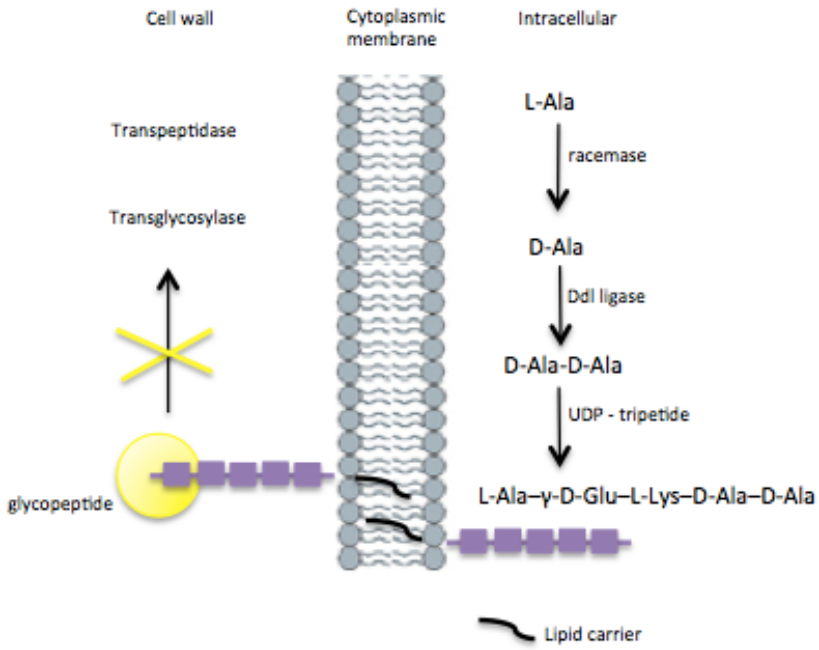


**Figure 5: Peptidoglycan biosynthesis and mechanism of action of vancomycin.**
Binding of the antibiotic to the C-terminal d-Ala–d-Ala of late peptidoglycan precursors prevents reactions catalyzed by transglycosylases and transpeptidases (69).

### 3.3.2 Therapeutic vancomycin drug monitoring

Some parameters either pharmacokinetic or pharmacodynamic were proposed to supervise vancomycin. These parameters including time (t), the concentration of vancomycin remains above the MIC (t>MIC), the ratio of the area under the serum drug concentration –versus- time curve and the MIC (AUC/MIC) and the ratio of the maximum serum drug concentration (Cmax) and the MIC (Cmax/MIC).

The parameter AUC/MIC was recommended by the reviews of pharmacokinetics and pharmacodynamics and a ratio ≥ 400 was defined as a target to obtain success with vancomycin (70). Nevertheless, is difficult to obtain various serum vancomycin concentrations to determine the AUC and applied this values in ratio AUC/MIC. So, the trough serum concentration monitoring is the most appropriate and practical technique to monitor the vancomycin (3, 70). It is recommended a total trough serum vancomycin concentration of 15–20 mg/L to try to increase clinical outcomes for complicated infections. That concentration

should obtain an AUC/MIC of 400 in a great number of patients if the MIC is 1 mg/L (70, 71).

Other important parameter to determine the success of a given dosage regimen is Vancomycin MIC. This value ideally should be provided by the clinical microbiology laboratory but the methodologies used to determine antimicrobial susceptibility (disk diffusion, automated microdilution, Etest method) limit the correct report of vancomycin MIC value by some laboratories (70).

### 3.3.3 Toxicity

Many of the adverse events attributed to vancomycin in the past were probably attributable to impurities present in older preparations that were present until the mid 1980s (4).

In the first trials was described an association between the usage of vancomycin and a little toxicity. Rash, chills and venous irritations were observed but believed to be related to the infusions. Ototoxicity was reported but considered to be related to elevated serum concentrations in individuals with renal failure (72). These problems were more frequent associated with the use of early preparations of vancomycin, therefore they were considered to be the result of impurities in the compound (3, 73, 74).

Infusion-related reactions associated with vancomycin have been well described and referred to as the “red man” syndrome. It is characterized by tingling or itch associated with flushing of the upper body. It usually occurs within a few minutes of the start of the infusion and resolves soon after cessation of the infusion. In cases where signs persist after cessation of the infusion or hypotension is prominent, hypersensitivity should be considered (71, 73).

Vancomycin rarely cases interstitial nephritis, it remains controversial whether modern preparations of vancomycin cause nephrotoxicity. Studies have used inconsistent definitions and had difficulty establishing the temporal relationship. They are also confounded by the use of other nephrotoxins, other potential causes of nephrotoxicity such as sepsis and underlying comorbidities. Several

studies have shown an association between higher trough concentrations (>15 mg/L) and nephrotoxicity, but it is unclear whether vancomycin is the cause or simply an indicator of renal toxicity (70). A consensus definition for toxicity has been proposed as repeated measured increases in creatinine >44 mmol/L or 50% above baseline after several days of vancomycin therapy in the lack of another reason. Is recommended that clinical approach first consider other causes and ensure adequate hydration before attributing nephrotoxicity to vancomycin (70).

### 3.3.4 Mechanisms of resistance to Vancomycin

The vancomycin activity is determined by specificity of substrate of the enzymes involved in structure of peptidoglycan precursors (69). Operons that encode enzymes are responsible for the resistance to vancomycin. First, with modifying the vancomycin-binding target with the synthesis of low-affinity precursors, in which the C-terminal d-Ala residue is replaced by d-lactate (d-Lac) or d-serine (d-Ser) and second removing the vancomycin-binding target, with elimination of the high-affinity precursors that are generally produced by the host (69).

High levels of resistance to vancomycin and teicoplanin characterize VanA-type resistance and it is presently, the only one detected in *S.aureus*. This resistance is based on modification of the vancomycin-binding target and is mediated by transposon Tn1546 that encodes 9 polypeptides which can be distributed in various functional groups: transposition (ORF1 and ORF2), regulation of resistance gene expression (VanR and VanS), synthesis of the d-Ala-d-Lac depsipeptide (VanH and VanA), and hydrolysis of peptidoglycan precursors (VanX and VanY) (69).

A dehydrogenase (VanH) reduces pyruvate to d-Lac and the VanA ligase catalyzes the formation of an ester bond between d-Ala and d-Lac. The d-Ala-d-Lac depsipeptide formed replaces the d-Ala-d-Ala dipeptide in peptidoglycan synthesis, a substitution that decreases the affinity of the molecule for glycopeptides. The removal of the susceptible precursors that terminate in d-Ala

prevents the interaction of vancomycin with its target (3, 69). Two enzymes are involved in this process, the VanX D,D-dipeptidase, which hydrolyzes the d-Ala-d-Ala dipeptide synthesized by the host d-Ala-d-Ala ligase (Ddl), and the VanY D,D-carboxypeptidase, which removes the C-terminal d-Ala residue of late peptidoglycan precursors when elimination of d-Ala-d-Ala by VanX is incomplete (figure 6) (69).

Conjugal transfer of plasmids that have acquired Tn1546-like elements by transposition seems to be responsible for the increase of glycopeptide resistance in enterococci (69). Either *in vitro* or *in vivo* it was possible transfer Van resistance genes from Enterococcus spp to S.aureus (75). In Michigan, Pennsylvania and New York were described MRSA isolates with high or moderate levels of resistance to vancomycin after acquisition of the vanA gene cluster (76-78).

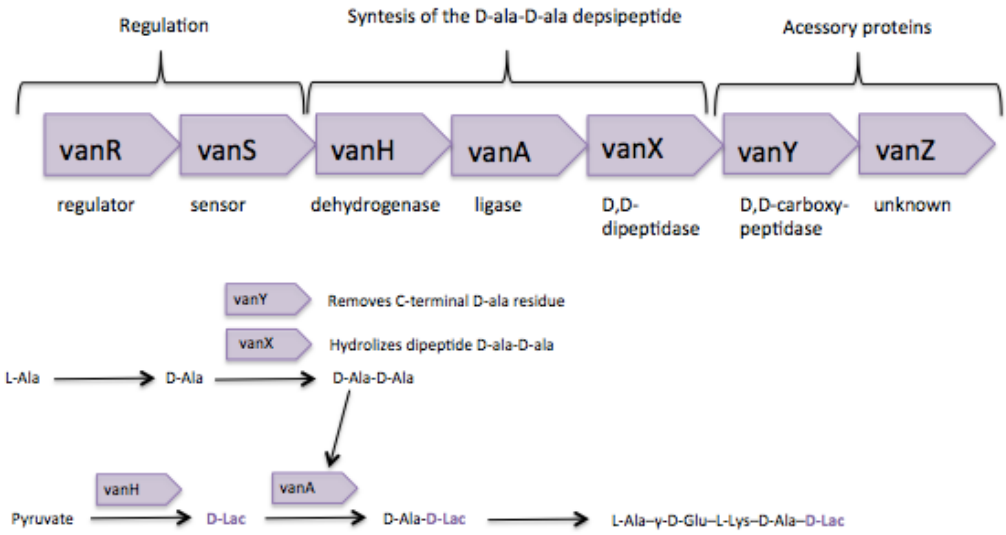


**Figure 6: VanA-type glycopeptide resistance.**

### 3.3.5 Vancomycin resistance and vancomycin MIC creep

Despite being approved for use in humans in 1958, vancomycin became an antibiotic of choice for treatment of MRSA infections in hospital settings only in the late 1980s (3).

In 1997, an *S.aureus* clinical isolate carried by a Japanese man was found to have a very low susceptibility to vancomycin. This isolate was the first reported Vancomycin Intermediate *S.aureus* (VISA) (79). VISA is typically associated with hospitalization, persistent infection, prolonged vancomycin treatment and/or treatment failure. The VISA phenotype is frequently preceded by an intermediate phenotype known in the clinical laboratory as heterogeneous VISA (hVISA) (67). An hVISA phenotype refers to a mixed cell population, derived originally from a single colony of *S.aureus*, in which the majority of cells have little or no resistance to vancomycin (MIC ≤ 2 mg/L) and a sub-population of cells is resistant to the antibiotic at the level of VISA (MIC ≥ 4 mg/L) (67).

Resistance to vancomycin was discovered in enterococci in the 1980s and this finding caused significant concern with regard to the future use of vancomycin as an effective treatment for MRSA (80). The first Vancomycin Resistant *S.aureus* (VRSA) isolate in the United States was reported in 2002 (81).

Complete vancomycin resistance in *S.aureus* is conferred by the vanA operon encoded on transposon Tn1546, originally a part of a vancomycin-resistant enterococci (VRE) conjugative plasmid [48]. *S.aureus* can acquire enterococcal plasmids during discrete conjugation events. Vancomycin resistance in *S.aureus* is maintained by retaining an original enterococcal plasmid or by a transposition of Tn1546 from the VRE plasmid into a staphylococcal resident plasmid. Risk factors for acquisition appear to be prior co-infection or colonization with MRSA and vancomycin resistant enterococci, substantial comorbidity and prolonged vancomycin use (82).

MRSA with decreased vancomycin susceptibility are categorized as heteroresistant, intermediate or resistant to vancomycin (83). This distinction has not, however, been applied by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) which defines all *S.aureus* strains with a vancomycin MIC of 4 mg/L as vancomycin resistant (60).

A phenomenon named “MIC creep” was described in recent years indicating a slow but stable increase in vancomycin MIC observed over time, from values of 0.5–0.75 mg/L to levels of 1.25–1.5 mg/L (4, 5). The visible growth in vancomycin MIC in MRSA isolates that was noticed in recent years can anticipate the appearance of isolates entirely resistant. The gradual increase in MICs to

vancomycin amongst susceptible *S.aureus* strains, are not the same in different parts of the world and are not necessarily universally present (82). Poor clinical outcomes, like delayed response, bigger rate of replace, extended duration of hospital admission or even mortality are being associated with patients with isolates that reveal MIC creep (6-8). The type of microbiological susceptibility test used (Etest, broth microdilution (BMD) or automated system), the type of *S.aureus* strain or the type of patient population evaluated can be compromising the results of MIC creep (6, 84). BMD continues to be the gold standard for measuring MIC (85). The literature is not consensual about this phenomenon, there are studies where vancomycin MIC creep is reported with BMD (85) and Etest methods (86), and studies that found no vancomycin MIC creep when using the same methods (87). The pool of publications related to MIC creep showed inconsistencies, with studies that report increases in the mean vancomycin MIC and studies that do not confirm these findings in MRSA.

## 3.4 Antimicrobial susceptibility testing in Microbiology Laboratory

The major responsibility of clinical microbiology laboratory is the evaluation of Antimicrobial Susceptibility Tests (AST).

*In vitro* AST are performed on pathogenic and/or resistant microorganisms. These tests are fundamental to monitor the resistance, epidemiological susceptibility and comparisons between new and existing agents (88).

Breakpoints defined by regulatory entities and professional groups are often the same. The Food and Drug Administration (FDA) and Clinical and Laboratory Standards Institute (CLSI), currently reviewed the breakpoints. In Europe, the MIC breakpoints have been defined by EUCAST (60).

### 3.4.1 Dilution methods for AST

The minimal antibiotics' concentration that is necessary to inhibit or ideally kill microorganisms can be obtained using dilution methods. Antibiotics are typically tested at 2-fold doubling ($log_2$) serial dilutions, with the lowest concentration of each antibiotic that inhibits visible growth of organisms designated as the MIC (89, 90).

Typically broth dilution testing include macro dilution, where the volumes of broth in test tubes for each dilution are typically ≥1 mL, and BMD, in which antimicrobial concentrations are most frequently of smaller volumes in 96-well microtiter plates. The BMD, is considered the gold standard method (88).

### 3.4.2 Agar disk diffusion test for AST

Mueller-Hinton agar is the medium recommended for routine testing, classifying isolates as susceptible, intermediate or resistant to antimicrobial agents. This method makes easier the identification of population heterogeneity (resistant subpopulations) and inoculum contamination ("mixed" cultures) because is more easily detected by agar than by broth testing methods (89).

In this testing method, prepared filter paper disks impregnated with specified predetermined concentrations of the antibiotics are applied to the surface of an agar medium previously inoculated with the microorganism. If the microorganism inoculated in the agar surface grows around the drugs diffused means that it is resistant to the antibiotic. In the other side, if the antibiotic inhibit the growth of the bacteria resulting in a inhibition zone around the drug means that the microorganism is susceptible to the antibiotic (88, 89).

The Etest method is used for determined the real MIC value in μg/ml, this method is based on quantitative gradient of concentration, allowing to determine the MIC between the conventional dilutions. Another advantage is that gradient of the drug on plastic strips remains stable for 18-24 hours, covering the critical times of the microorganisms growth (5).

### 3.4.3 Automated system

The automated system performs all the required steps to identify and determine the antimicrobial susceptibility profile after the preparation and standardization of the inoculum. This system reads the tests every 15 minutes and provides a kinetic analysis. The optical system combines multichannel fluorimeter and photometer readings to record fluorescence, turbidity, and colorimetric signals.
The results are interpreted by the database incorporated in the system. MICs of each antimicrobial agent are compared with MICs obtained by the reference method and the susceptibility is categorized as susceptible, intermediate, or resistant taking into account the current breakpoint criteria (91).

Considering the state of the art and the problem definition previously described, research objectives were assumed and the results obtained were described in the following section.

# Part II. Research

## 1 Objectives of research

1. Perform a meta-analysis on the prevalence of *mec*CMRSA, based on published studies and study the presence of suspect *mec*CMRSA in clinical isolates with a combination of two methodologies.

2. Evaluate the evidence of vancomycin MIC creep phenomenon recurring to a meta-analysis.

3. Assess vancomycin MIC distribution for *S.aureus* infections and to identify differences in vancomycin MIC through the application of different susceptibility testing methods.

The research developed resulted in three articles published in peer reviews journals and a fourth study is submitted to peer review and waiting approval (results will be presented during discussion – Part III).

# 2 Results

Study number 1: "Methicillin-resistant *Staphylococcus aureus* carrying the new *mec*C gene—a meta-analysis."

Results from the different studies do not provide a good overview of the real prevalence of *mec*C gene. With this knowledge, this study aimed to apply meta-analysis techniques to perform an improved evaluation of the available data on overall prevalence of *mec*CMRSA in humans and animals.

Study number 2: "Evaluation of vancomycin MIC creep in *Staphylococcus aureus* infections – a meta-analysis"

In an attempt to clarify inconsistencies in evaluation of MIC creep, this study intended to comprehensively assess the evidence of MIC creep, using a meta-analysis.

Study number 3: "Evaluation of vancomycin MIC creep in *Staphylococcus aureus* infections"

It is important to see the vancomycin MIC creep linked to a regional problem and the evaluation of their local susceptibility profiles should be essential in the local MRSA infection clinical management. This study assess the vancomycin MIC distribution for *S.aureus* infections, over a period of four years in Aveiro, Portugal.

Article **"Methicillin-resistant *Staphylococcus aureus* carrying the new *mec*C gene—a meta-analysis."**

Raquel Diaz, Elmano Ramalheira, Vera Afreixo, Bruno Gago

Diagnostic Microbiology and Infectious Disease, 84 (2016) 135-140

URL: http://www.dmidjournal.com/article/S0732-8893(15)00368-5/fulltext

DOI: 10.1016/j.diagmicrobio.2015.10.014

PMID: 26652130

Diagnostic Microbiology and Infectious Disease 84 (2016) 135–140

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Antimicrobial Susceptibility Studies

# Methicillin-resistant *Staphylococcus aureus* carrying the new *mec*C gene—a meta-analysis

Raquel Diaz [a,*], Elmano Ramalheira [b,1], Vera Afreixo [c,2], Bruno Gago [d,3]

[a] Health Sciences Department, University of Aveiro, Institute for Research in Biomedicine–iBiMED, Health Sciences Program, University of Aveiro, 3810-193 Aveiro, Portugal
[b] Health Sciences Department, University of Aveiro, Department of Medical Microbiology, Centro Hospitalar Baixo Vouga, Av. Artur Ravara, 3814-501, Aveiro, Portugal
[c] Department of Mathematics, University of Aveiro, Institute for Biomedicine–iBiMED, Health Sciences Program, University of Aveiro, 3810-193 Aveiro, Portugal
[d] Health Sciences Department, University of Aveiro, Institute for Biomedicine–iBiMED, Health Sciences Program, University of Aveiro, 3810-193 Aveiro, Portugal

ARTICLE INFO

*Article history:*
Received 9 June 2015
Received in revised form 10 September 2015
Accepted 19 October 2015
Available online 23 October 2015

*Keywords:*
*mec*C gene
*Staphylococcus aureus*
Prevalence

ABSTRACT

In 2011, a new *mec*A gene homolog, named *mec*C gene, was found in isolates from both humans and animals. The discovery of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the *mec*C gene has caused speculations about the origin, epidemiology, and impact of these isolates. The objective of this work is to perform a meta-analysis on the prevalence of *mec*C MRSA, based on previously published results. Meta-analysis showed that the overall pooled prevalence is 0.009% (95% confidence interval = 0.05–0.013) and that there was evidence of heterogeneity ($P < 0.01$, $I^2 = 97\%$). In conclusion, the very low reported prevalence provides an important baseline to monitor the epidemiology of this emerging form of MRSA.



## 1. Introduction

*Staphylococcus aureus* (*S. aureus*) was identified in the 1880s and is recognized as the most virulent species of staphylococci. Its versatility makes it a leading cause of morbidity and mortality, despite the large amount of effective antistaphylococcal antibiotics. *S. aureus* is a potentially pathogenic gram-positive bacteria causing infections that can go from minor impact, such as skin infection, to higher impact episodes such as postsurgical wound infections and systemic infections. (Dennis Kasper, 2010; Deurenberg and Stobberingh, 2008).

This microorganism is part of normal human flora; around 20% of individuals are colonized persistently nasally, and 30% are colonized intermittently. However, colonization can occur in many other locations such as the armpits, groin, and gastrointestinal tract (Williams et al., 1959). Colonization provides a reservoir from which bacteria can be introduced when host defenses are violated, for example during shaving, by inserting a catheter or during a surgery (Diekema et al., 2001; Wertheim et al., 2004). Colonization clearly increases the risk of subsequent infection in healthy individuals (Gordon and Lowy, 2008).

The vast majority of people who develop infections caused by *S. aureus* are infected with their own colonizing strains; however, these infections can also be obtained from other people or environmental exposures. Transmission results most frequently from transient colonization through the hands of hospital staff that carry strains from one patient to another (DeLeo et al., 2010; McCormick et al., 2001).

Resistance to antibiotics is a well-known serious problem in medicine. *S. aureus* has a great ability to acquire resistance to antibiotics. In 1961, Patricia Jevons reported the first isolates of methicillin-resistant *S. aureus* (MRSA), only 2 years after the clinical usage of methicillin. (Moellering, 2012; Sung et al., 2012)

In the following decades, MRSA isolates have spread throughout the world and can be found nowadays in many industrialized countries (DeLeo et al., 2010). For many years, the reported cases of MRSA infections were limited to hospitals, but the exponential emergence of community-associated MRSA (CA-MRSA) infections changed the epidemiology of *S. aureus* (Francois et al., 2004; Gerber et al., 2009) so that the distinction between CA-MRSA and hospital-associated MRSA is disappearing (Deurenberg and Stobberingh, 2008; Mediavilla et al., 2012; Sung et al., 2012).

### 1.1. Mechanism of resistance in MRSA

Pathogens are able to spread, establish ecological reservoirs, colonize, and cause disease. The mobile genetic elements involved in the spread of resistance and virulence in staphylococci are bacteriophages, chromosomal cassettes, plasmids, insertion sequences, and transposons (Hanssen and Ericson Sollid, 2006).

MRSA is resistant to practically all β-lactam antibiotics, and it is known that his resistance is caused by the production of a penicillin-

* Corresponding author. Tel.: +351-917-487-484.
*E-mail addresses:* raqueldiaz@ua.pt (R. Diaz), Elmano.ramalheira.11250@chbv.min-saude.pt (E. Ramalheira), vera@ua.pt (V. Afreixo), bmgago@ua.pt (B. Gago).
[1] Tel.: +351-234-892-085.
[2] Tel.: +351-234-370-213.
[3] Tel.: +351-234-247-247.

http://dx.doi.org/10.1016/j.diagmicrobio.2015.10.014
0732-8893/

binding protein (PBP) named PBP2A that reduces the binding affinities to β-lactam antibiotics. In methicillin-sensitive *S. aureus* (MSSA), β-lactam antibiotics bind to the native PBPs that are present in the *S. aureus* cell wall, which results in the disruption of the synthesis of the peptidoglycan layer causing *S. aureus* death (Fuda et al., 2004). On the other hand, in MRSA, PBP2A is present, and the binging of β-lactam antibiotics is not possible so that the synthesis of peptidoglycan layer occurs normally and with consequent MRSA colony development (Berger-Bachi and Rohrer, 2002; Deurenberg and Stobberingh, 2008). PBP2A is encoded by the gene *mec*A located in MRSA chromosome. Sequencing of the region containing *mec*A revealed a distinct mobile genetic element (SCC*mec*) that is present in MRSA but absent in MSSA. SCC*mec* elements are highly diverse, with 11 types (I–XI) recognized to date. (Katayama et al., 2000; Peacock and Paterson, 2015).

### 1.2. mecC gene in S. aureus

In 2011, a new *mec*A gene homolog, $mecA_{LGA251}$, was found in isolates from both humans and animals. The International Working Group on the Classification of Staphylococcal Cassette Chromosome (SCC) Elements renamed it to *mec*C (Ito et al., 2012).

The *mec*C gene shares 69% nucleotide homology with *mec*A and was found in a novel staphylococcal cassette chromosome (SCC) element designated as SCC*mec* type XI (Ito et al., 2012). Until now, isolates containing *mec*C gene have proved to be resistant phenotypically to β-lactams but have failed to be recognized as classical MRSA with PCRs for *mec*A, owing to different nucleotide composition (Kim et al., 2012; Shore et al., 2011; Stegger et al., 2012).

The discovery of MRSA carrying the *mec*C gene has caused speculations about the origin, epidemiology, and impact of these isolates. Several studies were implemented aiming at understanding this new gene present in *S. aureus* strains (Garcia-Alvarez et al., 2011). The detection, antimicrobial susceptibility testing, and treatment for *mec*C MRSA are not different from other MRSA strains. However, molecular techniques such as detection of MRSA with PCR or slide agglutination tests do not detect *mec*C MRSA. The use of these techniques, for primary detection or for confirmation of MRSA, can lead to false-negative results, and *S. aureus* will be erroneously diagnosed as methicillin susceptible (Garcia-Alvarez et al., 2011).

According to a study of Garcia-Alvarez et al. (2011), the discovery of *mec*C MRSA in dairy cows in England suggests that these animals might provide a reservoir of infection. Additionally, close links with farms or contact with dairy cattle could be risk factors that increase the likelihood of *mec*C MRSA carriage or infection in patients (Garcia-Alvarez et al., 2011). This study showed that *mec*C MRSA was resistant to penicillin, oxacillin, and cefoxitin but susceptible to gentamicin, neomycin, ciprofloxacin, tetracycline, erythromycin, clindamycin, fusidic acid, chloramphenicol, teicoplanin, rifampicin, trimethoprim, linezolid, and mupirocin. *mec*C MRSA showed negative reactions in the latex agglutination test for PBP2a, in PCR assays with primers for *mec*A and in PCR assays that amplify the SCC*mec*-orfX region of the SCC*mec*. Culture of *mec*C MRSA on agar plates with and without adjacent discs impregnated with both amoxicillin and clavulanic acid indicated that resistance was not mediated by β-lactamase hyperproduction (Garcia-Alvarez et al., 2011). The β-lactamase gene (blaZ) is present in SCC*mec*C, but the inability of clavulanate (a β-lactamase inhibitor) to change this resistance indicates that resistance is unlikely to be caused by β-lactamase. The most probable explanation for this resistance is the presence of *mec*A homologue in the type XI SCC*mec* (Garcia-Alvarez et al., 2011; Laurent et al., 2012).

The few available reports on this topic show that data on *mec*C MRSA in humans and animals are now becoming available, and relevant information on the epidemiology and prevalence of *mec*C MRSA can now be collected. Even so, at these point, results from the different studies do not provide a good overview of the current situation. The aim of this study is to apply meta-analysis techniques to perform an improved evaluation of the available data on overall prevalence of *mec*C MRSA in humans and animals.

## 2. Methods

### 2.1. Data collection

A review of the literature was conducted to identify epidemiological data about prevalence of *mec*C MRSA. The search strategy was limited to Medline database, English language, and articles published until April 2015. The keywords as search query used in PubMed engine were "methicillin-resistant Staphylococcus aureus", OR "methicillin-resistant Staphylococcus aureus [Mesh]" OR MRSA AND "*mec*C gene" OR "novel mecA" OR "new mecA" OR "LGA251".

The study hits from the search strategy were reviewed for inclusion and exclusion criteria. Studies were considered eligible for inclusion if it was possible to evaluate the prevalence of *mec*C MRSA in human and/or animal infections. Data extraction was guided by a checklist assessing clarity of aims and research questions.

The most relevant information was extracted from each eligible study (author, title, year and country, host species, sample size, and prevalence) and considered for the meta-analysis.

### 2.2. Statistical analysis

Homogeneity among studies was computed using the Cochran's Q statistic and the $I^2$ statistic. A significant Q statistic suggests that the distribution of effect sizes around the mean is greater than it would be predicted from sampling error alone, whereas the $I^2$ provides an estimate of the proportion of the variance in the aggregate effect size that is attributable to between-studies heterogeneity, with values of 0.25, 0.50, and 0.75 indicating low, moderate, and high degrees of heterogeneity (Johnson and Whisman, 2013).

In order to perform a secondary analysis, a subgroup analysis was carried out, and the estimated prevalence was reported for two subgroups: i) infections in humans and ii) infections in animals. Due to the significant heterogeneity between the studies, the pooled prevalence for each group was estimated using the random-effects model.

MetaXL 1.0., a tool for meta-analysis in Microsoft Excel, was used to pool individual prevalence from each study.

Publication bias was assessed using a funnel plot analysis. Funnel plots are scatter plots that plot effect size on the X axis and the standard error of the effect size on the Y axis and provide 95% confidence intervals (represented by cone-shaped lines) around the mean effect size. If only a few studies with negative or small effect sizes are included in the analysis or more than 5% of included studies fall outside of the confidence intervals, this will lead to asymmetry in the funnel plot and can indicate publication bias (Johnson and Whisman, 2013).

## 3. Results

According to the results of literature search, 248 articles published between January 2011 and April 2015 matched the searching criteria. From this group, 51 articles related to *mec*C gene in *S. aureus* and 37 studies on the screening of *mec*C gene were selected. Finally, studies with true prevalence of the *mec*C gene were included in the meta-analysis (Fig. 1 and Table 1).

The estimated prevalence in the human subgroup is 0.004% (95% confidence interval [CI] = 0.002–0.007), and the estimated prevalence in the animal subgroup is 0.10% (95% CI = 0.033–0.174). The overall estimated prevalence is 0.009% (95% CI = 0.005–0.013) (Fig. 2).

Publication bias was assessed using a funnel plot analysis. Visual inspection of a funnel plot can give an indication of publication bias. The studies are expected to spread symmetrically about the pooled effect size when publication bias is absent. Our study shows publication bias using funnel plot analysis (Fig. 3).

To evaluate the robustness of our analysis, we conducted a sensitivity analysis by recalculating the pooled results of the primary analysis by

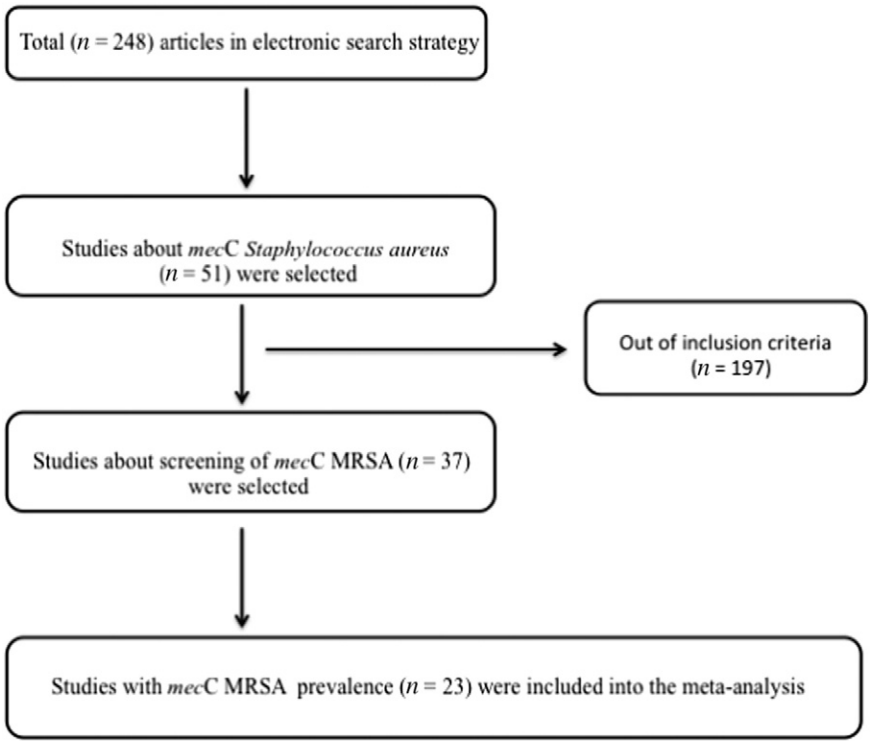


**Fig. 1.** Results of the literature search.

excluding each single study in turn. Table 2 revealed that the exclusion of any single study did not alter the overall combined result.

## 4. Discussion

In this work, 23 published studies from PubMed were identified as fulfilling the inclusion criteria, namely, of the availability of prevalence data on the presence of *mec*C MRSA in humans, animal, or both. Studies with true prevalence of the *mec*C gene were also included into the meta-analysis (Fig. 1 and Table 1), and studies with opportunist sampling, like that developed by Garcia-Alvarez et al. (2011), were excluded. In fact, the amount of proper prevalence studies is so reduced that the value of a meta-analysis cannot be really clear. However, it is the best statistical technique to evaluate *mec*C MRSA prevalence.

**Table 1**
Feature and characteristic studies included in study.

| Country | Host species | Sample size (*S. aureus*) | Period for the sampling | Prevalence | Studies references in meta-analysis (reference) |
|---|---|---|---|---|---|
| England | Human | 2010 | October 2011–June 2012 | 0.45 | Paterson et al. (2013) |
| USA | Human | 364 | June 2009–December 2011 | 0 | Ganesan et al. (2013) |
| Denmark | Human | 56382 | National collection of SAB isolates 1960–2011; national collection of MRSA 1988–2011; healthy nasal carriers 2009–2010; SAB received after August 2011 | 0.5 | Petersen et al. (2013) |
| | | 203 | August 2011–October 2011 | 5.9 | Stegger et al. (2012) |
| Germany | Human | 12691 | January 2006–June 2011 | 0.09 | Cuny et al. (2011) |
| | | 3207 | 2004–2005; 2010–2011 | 0.06 | Schaumburg et al. (2012) |
| | Animal | 38 | Unknown | 47.3 | Schlotter et al. (2014) |
| Belgium | Animal | 303 | August 2009–May 2011 | 0.99 | Vandendriessche et al. (2013) |
| | Human | 41 | August 2009–May 2011 | 0 | Vandendriessche et al. (2013) |
| | | 4869 | 2003–2012 | 0.18 | Deplano et al. (2014) |
| Spain | Human | 5505 | 2008–2013 | 0.04 | Garcia-Garrote et al. (2014) |
| | | 6 | January 2014 | 0 | Gomez et al. (2015) |
| | | 361 | 2009–2012 | 1.1 | Porrero et al. (2014) |
| | | 13 | 2011–2013 | 15 | Gomez et al. (2014) |
| | Animal | 16 | January 2014 | 68.8 | Gomez et al. (2015) |
| | | 601 | August 2008–July 2009 | 0.16 | Ariza-Miguel et al. (2014) |
| Netherlands | Animal | 55 | October 2011–January 2012 | 0 | van Duijkeren et al. (2014) |
| Switzerland | Human | 555 | 2005–2011 | 0 | Basset et al. (2013) |
| Austria | Human | 295 | 2009–2013 | 2.0 | Kerschner et al. (2014) |
| | Animal | 8 | Unknown | 37.5 | Loncaric et al. (2014) |
| Finland | Animal | 135 | 2003–2008 | 0.74 | Gindonis et al. (2013) |
| Sweden | Animal | 730 | 2010–2011 | 0.55 | Unnerstad et al. (2013) |
| Slovenia | Human | 395 | 2006–2013 | 1.5 | Dermota et al. (2015) |
| Jordan | Human | 56 | May 2011–April 2012 | 0 | Aqel et al. (2015) |
| France | Animal | 10 | 2011–2013 | 40 | Haenni et al. (2014) |

SAB = *S. aureus* bacteremia cases.

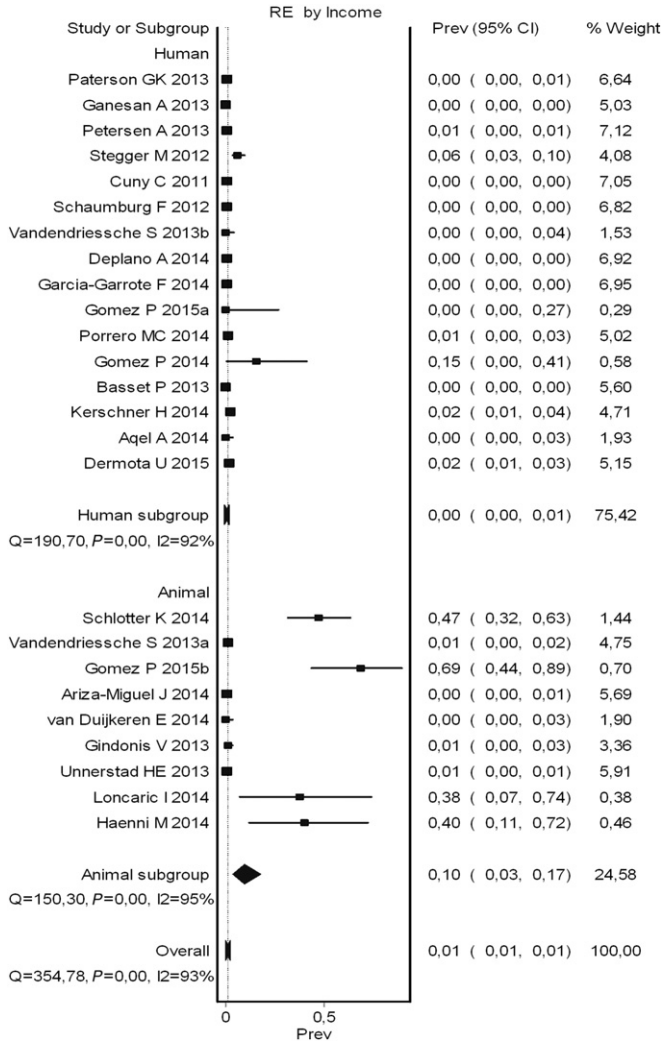


**Fig. 2.** Prevalence of *mec*C MRSA among studies included in meta-analysis.

At the time of this analysis, *mec*C MRSA was reported in 13 European countries and has been isolated from 14 host species (Paterson et al., 2014). MRSA harboring *mec*C has been reported from humans, livestock, domestic, and wild animals (Loncaric et al., 2014).

After discovery of the study of *mec*C gene, its prevalence became an important public health issue. In many studies, these reports represent small numbers of isolates identified by opportunistic sampling, like retrospective testing of previously identified atypical MRSA isolates. It is difficult to understand how common *mec*C MRSA truly is. The presence of *mec*C MRSA has been reported in animals and humans from several European countries, including Spain (Gomez et al., 2014), England

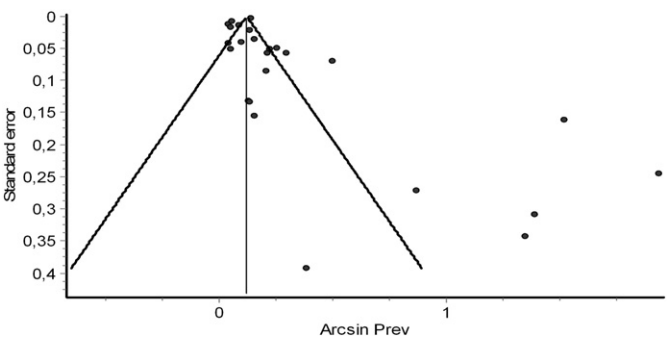


**Fig. 3.** Funnel plot analysis.

**Table 2**
Sensitivity analysis.

| Excluded study | Pooled prevalence | LCI 95% | HCI 95% | $I^2$ |
|---|---|---|---|---|
| Paterson et al. (2013) | 0.010 | 0.006 | 0.015 | 93.51 |
| Ganesan et al. (2013) | 0.010 | 0.006 | 0.015 | 93.48 |
| Petersen et al. (2013) | 0.011 | 0.006 | 0.018 | 91.83 |
| Stegger et al. (2012) | 0.008 | 0.004 | 0.012 | 92.93 |
| Cuny et al. (2011) | 0.011 | 0.006 | 0.017 | 92.32 |
| Schaumburg et al. (2012) | 0.010 | 0.006 | 0.015 | 93.24 |
| Schlotter et al. (2014) | 0.007 | 0.004 | 0.010 | 91.77 |
| Vandendriessche et al. (2013) | 0.009 | 0.005 | 0.014 | 93.47 |
| Vandendriessche et al. (2013) | 0.009 | 0.005 | 0.014 | 93.52 |
| Deplano et al. (2014) | 0.010 | 0.006 | 0.016 | 93.41 |
| Garcia-Garrote et al. (2014) | 0.010 | 0.006 | 0.015 | 92.77 |
| Gomez et al (2015) | 0.009 | 0.005 | 0.013 | 93.51 |
| Porrero et al (2014) | 0.009 | 0.005 | 0.013 | 93.45 |
| Gomez et al. (2015) | 0.009 | 0.005 | 0.013 | 93.38 |
| Gomez et al (2015) | 0.007 | 0.004 | 0.011 | 92.35 |
| Ariza-Miguel et al (2014) | 0.010 | 0.006 | 0.014 | 93.51 |
| van Duijkeren et al. (2014) | 0.009 | 0.005 | 0.014 | 93.52 |
| Basset et al. (2013) | 0.010 | 0.006 | 0.015 | 93.45 |
| Kerschner et al. (2014) | 0.008 | 0.005 | 0.013 | 93.35 |
| Gindonis et al (2013) | 0.009 | 0.005 | 0.014 | 93.50 |
| Unnerstad et al. (2013) | 0.009 | 0.006 | 0.014 | 93.50 |
| Aqel et al. (2014) | 0.009 | 0.005 | 0.014 | 93.52 |
| Loncaric et al. (2014) | 0.008 | 0.005 | 0.013 | 93.28 |
| Haenni et al. (2014) | 0.008 | 0.005 | 0.013 | 93.19 |
| Dermota et al. (2015) | 0.009 | 0.005 | 0.013 | 93.38 |

(Paterson et al., 2013), Denmark (Petersen et al., 2013), France (Haenni et al., 2014), Germany (Cuny et al., 2011), Sweden (Unnerstad et al., 2013), Belgium (Vandendriessche et al., 2013), Austria (Loncaric et al., 2014), and Finland (Gindonis et al., 2013) showing a wide geographical spread of these isolates. Several studies demonstrated that *mec*C MRSA is frequent in dairy cattle, suggesting that cows might provide a reservoir of infection. Gomez et al. (2015) found *mec*C MRSA in red deer in Southern Spain, suggesting that red deer could be acting as *mec*C MRSA hosts and could share these gene with other animals, such as small mammals when they coexist in the same habitat. In Sweden, Unnerstad et al. (2013) reported *mec*C MRSA in domestic animals suggesting them as potential reservoirs with associated risks to humans. Loncaric et al. (2014), in Austria, reported *mec*C MRSA in 2 bovine and 1 ovine from an organic farm. When wildlife and livestock share the same habitat, interspecies transmission can occur, either directly or indirectly. *mec*C MRSA was also found in urban wastewater, indicating that this gene can be expanding to new areas (Porrero et al., 2014). Even though the origin of *mec*C MRSA is unclear, there is evidence that contact with animals poses a zoonotic risk and that *mec*C MRSA can be transmitted between species (Paterson et al., 2014).

In humans, *mec*C gene has been predominantly described in skin and soft tissue infections, including fatal bacteremia. Garcia-Garrote et al. (2014) reported the first *mec*C MRSA human case in Spain, a patient with sepsis who died in the emergency department, highlighting the urgency of the implementation of testing methods in routine laboratory in order to rapidly detect and treat these emerging strains. The clinical impact of *mec*C MRSA is not fully determined. Isolates with MIC values to oxacillin/cefoxitin around the breakpoints should be regarded as suspect *mec*C MRSA demanding a respective *mec*C targeting assay (Becker et al., 2014).

Due to the significant heterogeneity between studies (see Cochran's Q and $I^2$ observed results in Fig. 2), random-effects model for meta-analysis was used in this study. The estimated prevalence in human subgroup is 0.004% (95% CI = 0.002–0.007), and the estimated prevalence of animal subgroup is 0.098% (95% CI = 0.033–0.174). The overall estimated prevalence is 0.009% (95% CI = 0.005–0.013).

The evaluation of *mec*C prevalence in humans and animals, to help monitoring the epidemiology of this emerging form of MRSA, is fundamental to stimulate the development of procedures that allow the correct diagnostic of *mec*C gene leading to adequate treatment decisions

when managing MRSA infections. This study shows that the overall estimated prevalence of *mec*C MRSA is still very low.

To evaluate the robustness of the estimate, 5 studies with highest prevalence were excluded (Gomez et al., 2014, 2015; Haenni et al., 2014; Loncaric et al., 2014; Schlotter et al., 2014). The overall prevalence presents significant differences from the obtained when all the studies were included in meta-analysis, and the estimated prevalence is 0.005. In these excluded studies, the authors used small samples of MRSA to screen *mec*C gene resulting in a high prevalence of this gene comparing with the other studies included in meta-analysis.

Sensitivity analysis by excluding 1 study at each turn and pooling results from the remainder further confirmed the robustness of our findings.

## 5. Conclusion

The discovery of the *mec*C gene may represent a public health threat since phenotypic and genotypic tests seem to be insufficient to detect this new resistance mechanism. The pool of results collected for this analysis indicates that it is important to study the prevalence of this gene in healthcare units to understand its dissemination and evolution over time and mainly to look for alternative treatments for infections caused by MRSA harboring this new gene. In this study, the overall estimated prevalence of *mec*C gene is 0.009% (95% CI = 0.005–0.013). Although the prevalence of *mec*C gene is extremely rare and only reported in European countries, diagnostic protocols, whether for clinical or epidemiological purposes, should consider the ramifications of not detecting *S. aureus* strains that carry this new *mec*A homolog.

## Funding

This study was supported by internal funding.

## Transparency declarations

None to declare.

## Acknowledgments

Mathematics Department, Health Sciences Department and Institute for Biomedicine–UID/BIM/04501/2013, University of Aveiro.

## References

Aqel AA, Alzoubi HM, Vickers A, Pichon B, Kearns AM. Molecular epidemiology of nasal isolates of methicillin-resistant Staphylococcus aureus from Jordan. J Infect Public Health 2015;8:90–7.

Ariza-Miguel J, Hernandez M, Fernandez-Natal I, Rodriguez-Lazaro D. Methicillin-resistant Staphylococcus aureus harboring mecC in livestock in Spain. J Clin Microbiol 2014;52:4067–9.

Basset P, Prod'hom G, Senn L, Greub G, Blanc DS. Very low prevalence of meticillin-resistant Staphylococcus aureus carrying the mecC gene in western Switzerland. J Hosp Infect 2013;83:257–9.

Becker K, Ballhausen B, Kock R, Kriegeskorte A. Methicillin resistance in Staphylococcus isolates: the "mec alphabet" with specific consideration of mecC, a mec homolog associated with zoonotic S. aureus lineages. Int J Med Microbiol 2014;304:794–804.

Berger-Bachi B, Rohrer S. Factors influencing methicillin resistance in staphylococci. Arch Microbiol 2002;178:165–71.

Cuny C, Layer F, Strommenger B, Witte W. Rare occurrence of methicillin-resistant Staphylococcus aureus CC130 with a novel mecA homologue in humans in Germany. PLoS One 2011;6(9):e22360.

DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant Staphylococcus aureus. Lancet 2010;375:1557–68.

Dennis Kasper AF. Staphylococcal Infections. In: Hill MG, editor. Harrison's infections diseases; 2010. p. 386–99.

Deplano A, Vandendriessche S, Nonhoff C, Denis O. Genetic diversity among methicillin-resistant Staphylococcus aureus isolates carrying the mecC gene in Belgium. J Antimicrob Chemother 2014;69:1457–60.

Dermota U, Zdovc I, Strumbelj I, Grmek-Kosnik I, Ribic H, Rupnik M, et al. Detection of methicillin-resistant Staphylococcus aureus carrying the mecC gene in human samples in Slovenia. Epidemiol Infect 2015;143:1105–8.

Deurenberg RH, Stobberingh EE. The evolution of Staphylococcus aureus. Infect Genet Evol 2008;8:747–63.

Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, et al. Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis 2001;15:S114–32.

Francois P, Renzi G, Pittet D, Bento M, Lew D, Harbarth S, et al. A novel multiplex real-time PCR assay for rapid typing of major staphylococcal cassette chromosome mec elements. J Clin Microbiol 2004;42:3309–12.

Fuda C, Suvorov M, Vakulenko SB, Mobashery S. The basis for resistance to beta-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant Staphylococcus aureus. J Biol Chem 2004;279:40802–6.

Ganesan A, Crawford K, Mende K, Murray CK, Lloyd B, Ellis M, et al. Evaluation for a novel methicillin resistance (mecC) homologue in methicillin-resistant Staphylococcus aureus isolates obtained from injured military personnel. J Clin Microbiol 2013;51:3073–5.

Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, et al. Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 2011;11:595–603.

Garcia-Garrote F, Cercenado E, Marin M, Bal M, Trincado P, Corredoira J, et al. Methicillin-resistant Staphylococcus aureus carrying the mecC gene: emergence in Spain and report of a fatal case of bacteraemia. J Antimicrob Chemother 2014;69:45–50.

Gerber JS, Coffin SE, Smathers SA, Zaoutis TE. Trends in the incidence of methicillin-resistant Staphylococcus aureus infection in children's hospitals in the United States. Clin Infect Dis 2009;49:65–71.

Gindonis V, Taponen S, Myllyniemi AL, Pyorala S, Nykasenoja S, Salmenlinna S, et al. Occurrence and characterization of methicillin-resistant staphylococci from bovine mastitis milk samples in Finland. Acta Vet Scand 2013;55:61.

Gomez P, Gonzalez-Barrio D, Benito D, Garcia JT, Vinuela J, Zarazaga M, et al. Detection of methicillin-resistant Staphylococcus aureus (MRSA) carrying the mecC gene in wild small mammals in Spain. J Antimicrob Chemother 2014;69:2061–4.

Gomez P, Lozano C, Gonzalez-Barrio D, Zarazaga M, Ruiz-Fons F, Torres C. High prevalence of methicillin-resistant Staphylococcus aureus (MRSA) carrying the mecC gene in a semi-extensive red deer (Cervus elaphus hispanicus) farm in Southern Spain. Vet Microbiol 2015;177:326–31.

Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant Staphylococcus aureus infection. Clin Infect Dis 2008;46(Suppl. 5):S350–9.

Haenni M, Chatre P, Tasse J, Nowak N, Bes M, Madec JY, et al. Geographical clustering of mecC-positive Staphylococcus aureus from bovine mastitis in France. J Antimicrob Chemother 2014;69(8):2292–3. http://dx.doi.org/10.1093/jac/dku130. [Epub 2014 Apr 28].

Hanssen AM, Ericson Sollid JU. SCCmec in staphylococci: genes on the move. FEMS Immunol Med Microbiol 2006;46:8–20.

Ito T, Hiramatsu K, Tomasz A, de Lencastre H, Perreten V, Holden MT, et al. Guidelines for reporting novel mecA gene homologues. Antimicrob Agents Chemother 2012;56:4997–9.

Johnson DP, Whisman MA. Gender differences in rumination: a meta-analysis. Pers Individ Dif 2013;55:367–74.

Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. Antimicrob Agents Chemother 2000;44:1549–55.

Kerschner H, Harrison EM, Hartl R, Holmes MA, Apfalter P. First report of mecC MRSA in human samples from Austria: molecular characteristics and clinical data. New Microbes New Infect 2014;3:4–9.

Kim C, Milheirico C, Gardete S, Holmes MA, Holden MT, de Lencastre H, et al. Properties of a novel PBP2A protein homolog from Staphylococcus aureus strain LGA251 and its contribution to the beta-lactam-resistant phenotype. J Biol Chem 2012;287:36854–63.

Laurent F, Chardon H, Haenni M, Bes M, Reverdy ME, Madec JY, et al. MRSA harboring mecA variant gene mecC, France. Emerg Infect Dis 2012;18:1465–7.

Loncaric I, Kubber-Heiss A, Posautz A, Stalder GL, Hoffmann D, Rosengarten R, et al. mecC- and mecA-positive meticillin-resistant Staphylococcus aureus (MRSA) isolated from livestock sharing habitat with wildlife previously tested positive for mecC-positive MRSA. Vet Dermatol 2014;25:147–8.

McCormick JK, Yarwood JM, Schlievert PM. Toxic shock syndrome and bacterial superantigens: an update. Annu Rev Microbiol 2001;55:77–104.

Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. Global epidemiology of community-associated methicillin resistant Staphylococcus aureus (CA-MRSA). Curr Opin Microbiol 2012;15:588–95.

Moellering Jr RC. MRSA: the first half century. J Antimicrob Chemother 2012;67:4–11.

Paterson GK, Morgan FJ, Harrison EM, Cartwright EJ, Torok ME, Zadoks RN, et al. Prevalence and characterization of human mecC methicillin-resistant Staphylococcus aureus isolates in England. J Antimicrob Chemother 2013;69(4):907–10.

Paterson GK, Harrison EM, Holmes MA. The emergence of mecC methicillin-resistant Staphylococcus aureus. Trends Microbiol 2014;22:42–7.

Peacock SJ, Paterson GK. Mechanisms of methicillin resistance in Staphylococcus aureus. Annu Rev Biochem 2015;84:577–601.

Petersen A, Stegger M, Heltberg O, Christensen J, Zeuthen A, Knudsen LK, et al. Epidemiology of methicillin-resistant Staphylococcus aureus carrying the novel mecC gene in Denmark corroborates a zoonotic reservoir with transmission to humans. Clin Microbiol Infect 2013;19(1):E16–22.

Porrero MC, Valverde A, Fernandez-Llario P, Diez-Guerrier A, Mateos A, Lavin S, et al. Staphylococcus aureus carrying mecC gene in animals and urban wastewater, Spain. Emerg Infect Dis 2014;20(5):899–901. http://dx.doi.org/10.3201/eid2005.130426.

Schaumburg F, Kock R, Mellmann A, Richter L, Hasenberg F, Kriegeskorte A, et al. Population dynamics among methicillin-resistant Staphylococcus aureus isolates in Germany during a 6-year period. J Clin Microbiol 2012;50:3186–92.

Schlotter K, Huber-Schlenstedt R, Gangl A, Hotzel H, Monecke S, Muller E, et al. Multiple cases of methicillin-resistant CC130 Staphylococcus aureus harboring mecC in milk and swab samples from a Bavarian dairy herd. J Dairy Sci 2014;97:2782–8.

Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, et al. Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent mecA, mecI, mecR1, blaZ, and ccr genes in human clinical isolates of clonal complex 130 methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2011; 55:3765–73.

Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, et al. Rapid detection, differentiation and typing of methicillin-resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mecA(LGA251). Clin Microbiol Infect 2012;18:395–400.

Sung JY, Lee J, Choi EH, Lee HJ. Changes in molecular epidemiology of community-associated and health care-associated methicillin-resistant Staphylococcus aureus in Korean children. Diagn Microbiol Infect Dis 2012;74:28–33.

Unnerstad HE, Bengtsson B, Horn af Rantzien M, Borjesson S. Methicillin-resistant Staphylococcus aureus containing mecC in Swedish dairy cows. Acta Vet Scand 2013;55:6.

van Duijkeren E, Hengeveld PD, Albers M, Pluister G, Jacobs P, Heres L, et al. Prevalence of methicillin-resistant Staphylococcus aureus carrying mecA or mecC in dairy cattle. Vet Microbiol 2014;171:364–7.

Vandendriessche S, Vanderhaeghen W, Soares FV, Hallin M, Catry B, Hermans K, et al. Prevalence, risk factors and genetic diversity of methicillin-resistant Staphylococcus aureus carried by humans and animals across livestock production sectors. J Antimicrob Chemother 2013;68:1510–6.

Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, et al. Risk and outcome of nosocomial Staphylococcus aureus bacteraemia in nasal carriers versus non-carriers. Lancet 2004;364:703–5.

Williams RE, Jevons MP, Shooter RA, Hunter CJ, Girling JA, Griffiths JD, et al. Nasal staphylococci and sepsis in hospital patients. Br Med J 1959;2:658–62.

Article **"Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections - a systematic review and meta-analysis"**

Raquel Diaz, Vera Afreixo, Elmano Ramalheira, Carmen Rodrigues, Bruno Gago
Clinical Microbiology and Infection (2017) 1 – 8
URL:
http://www.clinicalmicrobiologyandinfection.com/article/S1198743X(17)30337-3/fulltext
DOI: 10.1016/j.cmi.2017.06.017
PMID: 28648858

Clinical Microbiology and Infection xxx (2017) 1–8

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Systematic review

# Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections—a systematic review and meta-analysis

R. Diaz [1, 2, *], V. Afreixo [2, 3, 4], E. Ramalheira [1, 5], C. Rodrigues [3], B. Gago [1, 2]

[1] *Department of Medical Sciences, University of Aveiro, Aveiro, Portugal*
[2] *Institute of Biomedicine—iBiMED, University of Aveiro, Aveiro, Portugal*
[3] *Department of Mathematics, University of Aveiro, Aveiro, Portugal*
[4] *CIDMA—Centre for Research & Development in Mathematics and Applications, Aveiro, Portugal*
[5] *Department of Medical Microbiology, Centro Hospitalar, Aveiro, Portugal*

ARTICLE INFO

*Article history:*
Received 11 February 2017
Received in revised form
29 May 2017
Accepted 1 June 2017
Available online xxx

Editor: L. Leibovici

*Keywords:*
Meta-analysis
MIC creep
MIC methodologies
*Staphylococcus aureus*
Vancomycin

ABSTRACT

*Objectives:* Vancomycin is currently the primary option treatment for methicillin-resistant *Staphylococcus aureus* (MRSA). However, an increasing number of MRSA isolates with high MICs, within the susceptible range (vancomycin MIC creep), are being reported worldwide. Resorting to a meta-analysis approach, this study aims to assess the evidence of vancomycin MIC creep.
*Methods:* We searched for studies in the PubMed database. The inclusion criteria for study eligibility included the possibility of retrieving the reported data values of vancomycin MIC and information concerning the applied MIC methodology.
*Results:* The mean values of vancomycin MICs, of all 29 234 *S. aureus* isolates reported in the 55 studies included in the meta-analysis, were 1.23 mg/L (95% CI 1.13−1.33) and 1.20 mg/L (95% CI 1.13−1.28) determined by Etest and broth microdilution method, respectively. No significant differences were observed between these two methodologies. We found negative correlation between pooled mean/pooled proportion and time strata.
*Conclusions:* We have found no evidence of the MIC creep phenomenon. **R. Diaz, Clin Microbiol Infect 2017;▪:1**


## Introduction

*Staphylococcus aureus* is one of the most common pathogens causing severe infections [1]. Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are associated with increased morbidity, longer antibiotic therapy, higher healthcare costs, prolonged hospitalization and increased risk of death [2].

The first option for the treatment of invasive MRSA infections is the glycopeptide vancomycin, which continues to be the reference standard approach in this context [3]. The use of vancomycin has been increasing since the mid-1980s, resulting in the emergence of MRSA with reduced susceptibility to vancomycin [2]. Recently, a phenomenon of gradual increase in the value of glycopeptide MIC for *S. aureus* was observed and reported in the literature as MIC creep [4]. The publications related to MIC creep included studies that report increases in the mean vancomycin MIC as well as studies that did not confirm these findings in MRSA [3,5,6]. In this context, and as a result of the reported vancomycin therapy failure in patients with *S. aureus* infections with a MIC ≥4 mg/L, the CLSI reduced vancomycin breakpoints from ≤4 mg/L to ≤2 mg/L, for susceptible *S. aureus*, and from ≥32 mg/L to ≥16 mg/L for resistant *S. aureus*. These changes aimed to increase the sensitivity of the detection of non-susceptible isolates [3,7]. The apparent increase in vancomycin MIC among MRSA, observed in recent years, can represent the first step towards the appearance of fully resistant isolates. Patients infected with MRSA isolates that exhibit MIC creep might experience poorer clinical outcomes, including delayed treatment response, increased mortality, increased rate of relapse, extended hospitalization or overall increased cost of hospitalization [2,7,8].

* Corresponding author. R. Diaz, Department of Medical Sciences, University of Aveiro, Institute of Biomedicine—iBiMED, University of Aveiro, 3810-193 Aveiro, Portugal.
*E-mail address:* raqueldiaz@ua.pt (R. Diaz).

http://dx.doi.org/10.1016/j.cmi.2017.06.017
1198-743X/

Soriano *et al.* showed that mortality associated with MRSA bacteraemia was significantly higher when the empirical antibiotic was inappropriate and when vancomycin was empirically used for the treatment of infection with strains with high vancomycin MIC (>1 mg/L) [9].

The MIC creep phenomenon may be influenced by the type of microbiological susceptibility test used (Etest, broth microdilution (BMD) or automated system) [2,10], type of *S. aureus* strain or type of patient population evaluated [2]. The reference standard for measuring MIC remains BMD [11].

In the literature it is possible to find studies that show vancomycin MIC creep by using BMD [12] and Etest methods [13], and studies that found no vancomycin MIC creep when using the same methods [12–14]. Such inconsistent information about the MIC creep phenomenon and conflicting results have been noted in cases in which MIC creep could not be detected. Hence, in an attempt to clarify these inconsistencies and conflicting results, this study aims to assess the evidence of MIC creep, using a meta-analysis, highlighting the type of applied MIC methodologies.

## Materials and methods

### *Search strategy and selection criteria*

The studies to be included in the meta-analysis approach were retrieved from the PubMed database. The search query was 'methicillin-resistant *staphylococcus aureus*' OR 'Methicillin-Resistant Staphylococcus aureus'[Mesh]" OR 'MRSA' AND 'vancomycin' OR 'vancomycin'[Mesh]" AND 'minimal inhibitory concentration' OR 'MIC' OR 'MIC creep' OR 'reduced vancomycin susceptibility' OR 'vancomycin susceptibility trends'.

The abstracts of the collected articles were reviewed and a study was considered to be eligible for inclusion if it included values of vancomycin MIC and details of the applied MIC methodologies. Selected MIC methodologies were: microdilution (BMD), Etest, agar diffusion and automated systems.

### *Data analysis*

#### *Data extraction*

After the analysis of both titles and abstracts, the selected studies were independently assessed and analysed by three authors (R. Diaz, V. Afreixo and C. Rodrigues).

Data extracted from the identified studies included MIC vancomycin information applied methodology, number of studied isolates, source of isolate, year of study completion and country (see Supplementary material, Data S1).

#### *Data uniformization*

In the cases of the studies that only reported median, minimum (min) and maximum (max) values, it was assumed that the symmetry and mean values were estimated from the median. To estimate the standard deviation (SD), the authors assumed uniformity with a variable distribution and the $SD = (max - min)/\sqrt{12}$. To complete the table of statistics (mean, SD, min), all MIC values $\leq 0.5$ were converted to 0.5.

In studies in which the results were grouped by periods of $\geq 2$ years, the counts were divided in a uniform manner by periods under review.

To create groups with similar numbers of effects, the following stratification of study years under analysis was selected, resulting in seven time strata: <2000; 2000–2001; 2002–2003; 2004–2005; 2006–2007; 2008–2009 and ≥2010. The time strata <2000 and ≥2010 are created to reach a more uniform number of studies in each stratum. To aggregate results from different years, from the same study, weighted averages, combined variances and accumulated frequencies were used.

Some criteria were defined before starting the meta-analysis on the 53 included studies: (a) in studies that presented data of frozen isolates and data of 'at time' isolates, data of 'frozen isolates' were used because most of the studies determined MICs in 'frozen isolates' [6]; (b) in the case of studies with data of automated methods, only data of the VITEK method were considered as it is the only method common to all studies [15]; (c) studies of the same author and year were identified with A and B [15–18]; (d) studies that included different cities were identified with A and B [3]; (e) data presented in more than one study of the same author were excluded [17,18].

### *Statistical analysis*

Homogeneity among studies was computed using the Cochran's $Q$ statistic and the $I^2$ statistic. A significant $Q$ statistic suggests that the distribution of effect sizes around the mean is greater than it would be predicted from sampling error alone. On the other hand, $I^2$ provides an estimate of the proportion of the variance in the aggregate effect size that is attributable to study heterogeneity, with values of 0.25, 0.50 and 0.75 indicating low, moderate and high degrees of heterogeneity.

To perform a secondary study, a subgroup analysis was carried out with the mean of vancomycin MIC and the proportion of *S. aureus* isolates with vancomycin MIC ≥2 mg/L in four subgroups of regions: (a) Europe, (b) USA, (c) Asia and (d) others. Due to the significant heterogeneity between the studies, the pooled prevalence for each group was estimated using the random-effects model.

To compare the pooled effect size in different groups (subgroups) the $Z$-test was used and for simultaneous statistical tests the Sidak correction was applied [19].

MetaXL 1.0, a tool for meta-analysis in Microsoft Excel, was used to pool individual prevalence from each study.

## Results

Literature search, based on the keywords described in the Materials and methods section, identified 980 studies (Fig. 1). After title and abstract analysis, 880 were excluded and 100 full-text articles were reviewed [1,3–6,8,9,11–18,20–104]. Of these, 55 studies were included in the meta-analysis (see Supplementary material, Table S1), 45 were excluded for the following reasons: data were included in another study of the same author (two studies) [87], no MIC data were available (35 studies) [21,26,27,30,31,33,35,37,38,41,44,47,48,53,54,59,64,69,70,78,80,82,83,89,90,92,94,95,97,101,102,104], full-text versions were unavailable (two studies) [75,77], different guidelines were used (two studies) [47,103] and meta-analysis and review articles (four studies) [8,46,57,93].

Considering all studies included in the pool ($n = 55$), the mean vancomycin MIC was 1.20 mg/L (95% CI 1.13–1.28), 1.23 mg/L (95% CI 1.13–1.33) and 1.19 mg/L (95% CI 1.07–1.30), when determined by the BMD method, Etest method and agar method, respectively. The pooled mean of vancomycin MIC determined resorting to the automated method was lower compared with values obtained with the other methods (1.10 mg/L) (Table 1). The differences between studied MIC methodologies were not statistically significant ($p > 0.05$, $Z$-test with Sidak correction for multiple comparisons).

To evaluate the robustness of our uniformization, sensitivity analysis was conducted. In four studies the mean value was inferred

Please cite this article in press as: Diaz R, et al., Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections—a systematic review and meta-analysis, Clinical Microbiology and Infection (2017), http://dx.doi.org/10.1016/j.cmi.2017.06.017

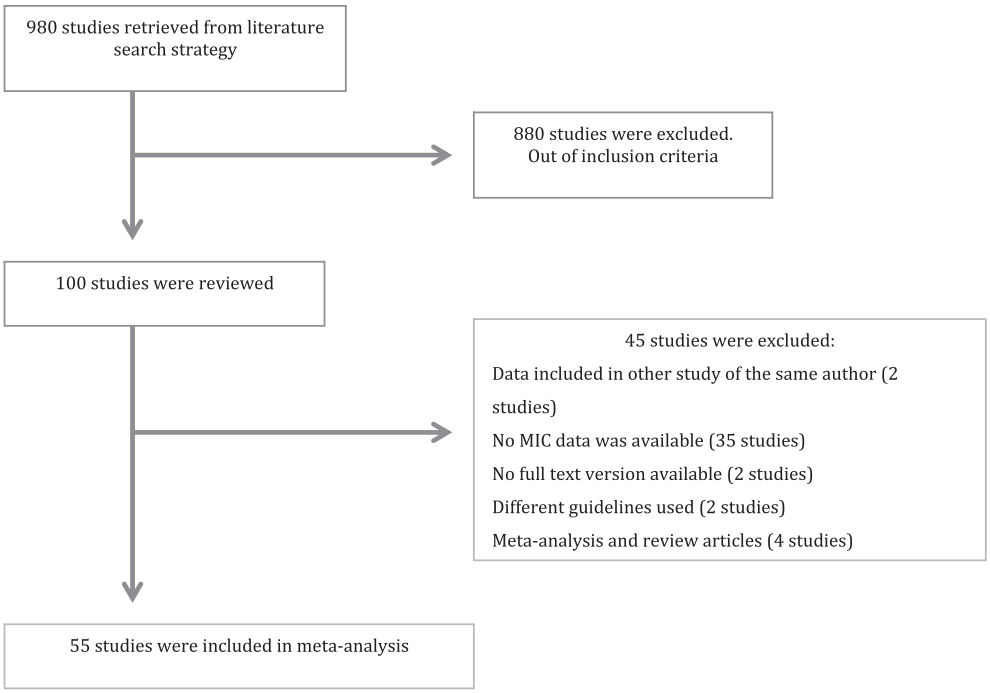


**Fig. 1.** Results of literature search.

**Table 1**
Statistical results of pooled mean vancomycin MICs determined resorting to different MIC testing methodologies

| | MIC testing methodologies | Pooled mean | 95% CI | | Spearman correlation[a] | $I^2$ | Number of independent studies | Number of samples |
|---|---|---|---|---|---|---|---|---|
| | | | Lower bound | Upper bound | | | | |
| MRSA | BMD | 1.20 | 1.13 | 1.28 | −0.82 | 98.69 | 16 | 8328 |
| | Etest | 1.23 | 1.13 | 1.33 | −0.57 | 99.61 | 27 | 7426 |
| | Agar | 1.19 | 1.07 | 1.30 | — | 97.64 | 6 | 1626 |
| | Automated system | 1.10 | 0.83 | 1.38 | — | 99.24 | 7 | 1555 |

[a] Spearman correlation: correlation between time strata and pooled mean.

through the median [3,24,40,79], excluding these studies the overall results do not present significant differences, the overall results are similar with negative correlation values, −0.68 and −0.64 for Etest and BMD, respectively.

Considering the pooled mean of vancomycin MIC over each time stratum represented in Figs. 2 and 3, studies before the year 2000 showed the highest vancomycin MICs pooled values. After the year 2007 vancomycin MIC showed a slight decrease (negative Spearman correlation between time strata and pooled mean) (Table 2). In general, the results were similar for BMD (Fig. 2) and Etest (Fig. 3) methods.

The CLSI guidelines consider *S. aureus* to be susceptible to vancomycin for MICs <2 mg/L [105]. Considering this guideline, the pooled proportion of MRSA isolates with vancomycin MIC ≥2 mg/L was evaluated and shown to be low, between 14% and 18%, for all the applied testing methods (Table 2).

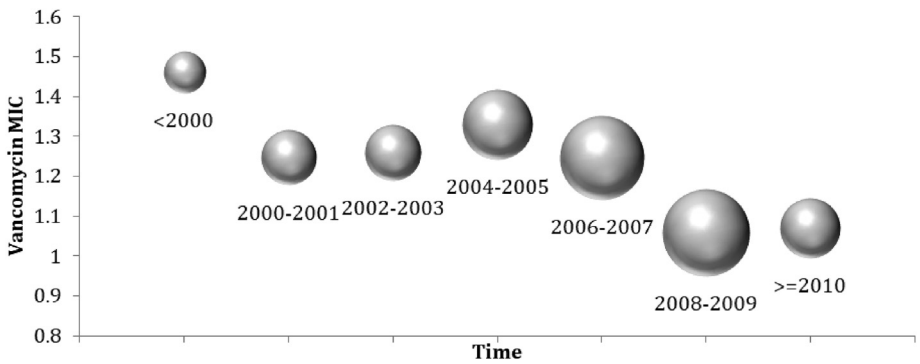


**Fig. 2.** Pooled mean of vancomycin MIC determined by the broth microdilution method over time. The bubble size represents the meta-analysis sub-group weight.

Please cite this article in press as: Diaz R, et al., Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections—a systematic review and meta-analysis, Clinical Microbiology and Infection (2017), http://dx.doi.org/10.1016/j.cmi.2017.06.017

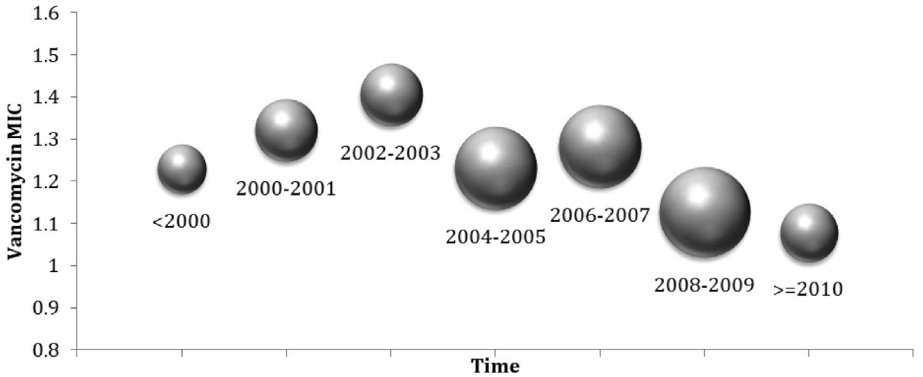


**Fig. 3.** Pooled mean vancomycin MIC determined by the Etest method over time. The bubble size represents the meta-analysis sub-group weight.

**Table 2**
Pooled proportion of the *Staphylococcus aureus* isolates with vancomycin MIC ≥2 mg/L determined using to different MIC testing methodologies

| | MIC testing methodologies | Pooled proportion | 95% CI | | Spearman correlation[a] | $I^2$ | Number of independent studies | Number of samples |
|---|---|---|---|---|---|---|---|---|
| | | | Lower bound | Upper bound | | | | |
| MRSA | Broth microdilution | 0.18 | 0.12 | 0.25 | −0.89 | 98.48 | 17 | 10 350 |
| | Etest | 0.14 | 0.10 | 0.19 | −0.64 | 96.86 | 27 | 7389 |
| | Agar | 0.15 | 0.04 | 0.30 | — | 98.32 | 7 | 2016 |
| | Automated system | 0.18 | 0.05 | 0.36 | — | 97.99 | 6 | 1406 |

[a] Spearman correlation: correlation between time strata and pooled proportion.

The analysis of the distribution of MRSA isolates with vancomycin MIC ≥2 mg/L showed a decrease over time, with either BMD (Fig. 4) or Etest (Fig. 5) methods. For the Etest method, a slight oscillation was observed between 2000 and 2007, followed by a tendency to decrease after 2007 (negative Spearman correlation) (Table 2).

Regarding the analysis of the pooled mean of vancomycin MIC by region, (Europe, USA, Asia and other countries), in Europe the pooled mean of vancomycin MIC determined with the BMD method was 1.12 mg/L (see Supplementary material, Fig. S1) and with the Etest method was 1.13 mg/L (see Supplementary material, Fig. S2). In Asia, the pooled mean of vancomycin determined with the BMD method was 1.17 mg/L (see Supplementary material, Fig. S1) and with the Etest method was 0.98 mg/L (see Supplementary material, Fig. S2). Regarding the USA, these values were slightly increased, with values of 1.37 mg/L and 1.53 mg/L for the BMD method and Etest method, respectively (see Supplementary material, Figs. S1 and S2).

By region, the proportion of MRSA isolates with vancomycin MIC ≥2 mg/L was 17% in Europe, 26% in the USA and 18% in Asia, for the BMD method. For the Etest method the proportion of MRSA isolates was 11% in Europe, 27% in USA and 3% in Asia (Table 3).

## Discussion

This study is the first meta-analysis with a worldwide perspective that evaluates the trends of vancomycin MIC over time, determined by different MIC methodologies, and no statistically significant evidence of the MIC creep phenomenon was detected.

The standard for measuring MIC remains BMD; but this is a labour intensive technique and many laboratories use the Etest method as an alternative. These two methods were selected for evaluation in more detail in this work.

The results of the pooled means of vancomycin MICs for all MRSA isolates reported with BMD and Etest methods were 1.20 mg/L and 1.23 mg/L, respectively, and no significant differences were

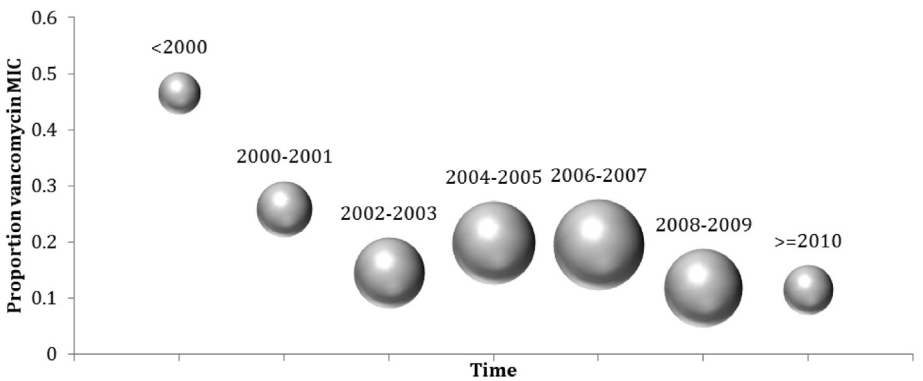


**Fig. 4.** Pooled proportion of methicillin-resistant *Staphylococcus aureus* isolates with vancomycin MIC ≥2 mg/L determined with the broth microdilution method. The bubble size represents the meta-analysis sub-group weight.

Please cite this article in press as: Diaz R, et al., Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections—a systematic review and meta-analysis, Clinical Microbiology and Infection (2017), http://dx.doi.org/10.1016/j.cmi.2017.06.017

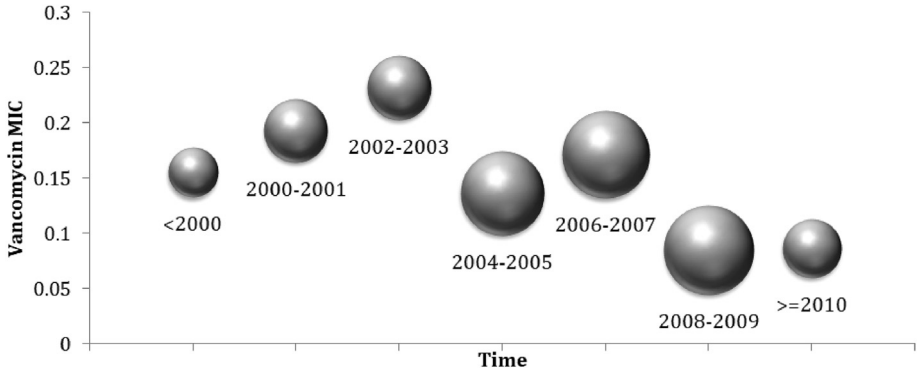


**Fig. 5.** Pooled proportion of methicillin-resistant *Staphylococcus aureus* isolates with vancomycin MIC ≥2 mg/L determined with the Etest method. The bubble size represents the meta-analysis sub-group weight.

**Table 3**
Pooled proportion of the methicillin-resistant *Staphylococcus aureus* isolates with vancomycin MIC ≥2 mg/L, by region

| MIC testing methodologies | Region | Pooled | 95% CI | | $I^2$ | Number of independent studies | Number of samples |
|---|---|---|---|---|---|---|---|
| | | | Lower bound | Upper bound | | | |
| Broth microdilution | Europe | 0.17 | 0.06 | 0.31 | 99.58 | 5 | 1585 |
| | USA | 0.26 | 0.00 | 0.71 | | 4 | 4518 |
| | Asia | 0.18 | 0.00 | 0.44 | | 4 | 3235 |
| | Other | 0.10 | 0.00 | 0.42 | | 2 | 435 |
| Etest | Europe | 0.11 | 0.04 | 0.20 | 98.92 | 10 | 1730 |
| | USA | 0.27 | 0.11 | 0.46 | | 11 | 4578 |
| | Asia | 0.03 | 0.00 | 0.09 | | 5 | 987 |
| | Other | 0.23 | 0.00 | 0.65 | | 2 | 435 |

observed between these two methods. The number of strains studied with the agar method was very low when compared with other methodologies; however, the pooled mean of vancomycin MIC for this method (1.19 mg/L) was not significantly different from the others. Based on clinical laboratory practice, as expected, the pooled mean of vancomycin MIC determined with an automated method (1.10 mg/L) was lower than those determined with other methodologies studied. This result is consistent with the study of Tomczak *et al.*, which reports differences between vancomycin MIC assayed with an automated method and Etest method [4].

When considering pooled mean of vancomycin MIC over time, studies published before the year 2000 exhibited the highest vancomycin MICs and after 2007 vancomycin MICs showed a slight decrease. Results were similar for BMD and Etest methods. These findings are consistent with the results reported previously by other authors that did not find trends in vancomycin MIC. Some examples are the SENTRY Antimicrobial Surveillance Program database, where, between 1998 and 2003, 35 458 *S. aureus* isolates where studied [106], a multicentre study of nine US medical centres where 1800 MRSA samples where studied, between 1999 and 2006 [107], and a survey from Spain that, between 2002 and 2006, evaluated 3141 *S. aureus* isolates [12].

Considering the upper vancomycin breakpoint for susceptible *S. aureus*, a subgroup analysis was carried with the proportion of *S. aureus* isolates with vancomycin MIC ≥2 mg/L; comparing the two main testing methods under analysis the MRSA pooled proportion was very low (11%–27%). Additionally, over time strata, both BMD and Etest methods showed a decrease in vancomycin MIC, strengthening the observation of no evidence of MIC creep, as supported by the Spearman's correlation coefficient (Table 2). The decrease trends observed in both analyses for the last time-strata, can suggest a positive impact of implementation of more rigorous clinical strategies for the management of MRSA infection.

This study also enabled the evaluation of the pooled mean of vancomycin MIC by region (see Supplementary material, Figs. S1 and S2). The results showed a lower pooled mean of vancomycin MIC in Europe and a slightly higher pooled mean of vancomycin MIC in the USA. It is expected that an increased value of vancomycin MIC is related to the overall prevalence of MRSA, with a higher value of vancomycin MIC linked to higher MRSA prevalence. When correlating our results with the overall prevalence of countries included in the meta-analysis, this can be found in the USA and China where the overall prevalence of MRSA is 55.9% [56] in the USA (with a pooled mean of vancomycin 1.12 mg/L and 1.13 mg/L, determined with BMD and Etest, respectively) and 46.8% in China [56] (with a pooled mean of vancomycin 1.17 mg/L for BMD and 0.98 mg/L for Etest).

One of the problems of combining data from multiple centres is that it can obscure trends that may exist within a given institution or country, because of differences in patient populations and drug-usage patterns. One possible limitation of our study is the inclusion of large multicentre studies, but the negative values obtained with Spearman's correlation coefficient even with inclusion of these studies, substantiate no evidence of vancomycin MIC creep over time.

The present study did not detect an increase in vancomycin MIC, suggesting that vancomycin continues to be the standard option in treatment of MRSA infections when MIC is determined with Etest or BMD methods and in institutions that continuously evaluate their local susceptibility profiles. Future studies must focus on the analysis of vancomycin MIC creep on a regional basis, tested at the same locations and using the same methodologies.

## Transparency declarations

None to declare.

## Funding

This study was supported by internal funding.

Please cite this article in press as: Diaz R, et al., Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections—a systematic review and meta-analysis, Clinical Microbiology and Infection (2017), http://dx.doi.org/10.1016/j.cmi.2017.06.017

## Acknowledgements

This work was supported by Portuguese funds through the iBiMED – Institute of Biomedicine, IEETA – Institute of Electronics and Telematics Engineering of Aveiro and the Portuguese Foundation for Science and Technology ("FCT—Fundação para a Ciência e a Tecnologia"), within project COMPETE/FEDER UID/BIM/04501/2013 and UID/MAT/04106/2013.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2017.06.017.

## References

[1] Yeh YC, Yeh KM, Lin TY, Chiu SK, Yang YS, Wang YC, et al. Impact of vancomycin MIC creep on patients with methicillin-resistant *Staphylococcus aureus* bacteremia. J Microbiol Immunol Infect 2012;45:214–20.
[2] Dhand A, Sakoulas G. Reduced vancomycin susceptibility among clinical *Staphylococcus aureus* isolates ('the MIC Creep'): implications for therapy. F1000 Med Rep 2012;4:1.
[3] Kehrmann J, Kaase M, Szabados F, Gatermann SG, Buer J, Rath PM, et al. Vancomycin MIC creep in MRSA blood culture isolates from Germany: a regional problem? Eur J Clin Microbiol Infect Dis 2011;30:677–83.
[4] Tomczak H, Szalek E, Blazejewska W, Myczko K, Horla A, Grzeskowiak E. The need to assay the real MIC when making the decision to eradicate *Staphylococcus aureus* with vancomycin. Postepy Hig Med Dosw (Online) 2013;67:921–5.
[5] Ho PL, Lo PY, Chow KH, Lau EH, Lai EL, Cheng VC, et al. Vancomycin MIC creep in MRSA isolates from 1997 to 2008 in a healthcare region in Hong Kong. J Infect 2010;60:140–5.
[6] Edwards B, Milne K, Lawes T, Cook I, Robb A, Gould IM. Is vancomycin MIC "creep" method dependent? Analysis of methicillin-resistant *Staphylococcus aureus* susceptibility trends in blood isolates from North East Scotland from 2006 to 2010. J Clin Microbiol 2012;50:318–25.
[7] Rossatto FC, Proenca LA, Becker AP, Silveira AC, Caierao J, D'Azevedo PA. Evaluation of methods in detecting vancomycin MIC among MRSA isolates and the changes in accuracy related to different MIC values. Rev Inst Med Trop Sao Paulo 2014;56:469–72.
[8] Kalil AC, Van Schooneveld TC, Fey PD, Rupp ME. Association between vancomycin minimum inhibitory concentration and mortality among patients with *Staphylococcus aureus* bloodstream infections: a systematic review and meta-analysis. JAMA 2014;312:1552–64.
[9] Soriano A, Marco F, Martinez JA, Pisos E, Almela M, Dimova VP, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. Clin Infect Dis 2008;46:193–200.
[10] Himani, Agrawal C, Madan M, Pandey A, Thakuria B. Methicillin resistant *Staphylococcus aureus*: inconsistencies in vancomycin susceptibility testing methods, limitations and advantages of each method. J Clin Diagnos Res 2015;9:DC01–4.
[11] van Hal SJ, Barbagiannakos T, Jones M, Wehrhahn MC, Mercer J, Chen D, et al. Methicillin-resistant *Staphylococcus aureus* vancomycin susceptibility testing: methodology correlations, temporal trends and clonal patterns. J Antimicrob Chemother 2011;66:2284–7.
[12] Alos JI, Garcia-Canas A, Garcia-Hierro P, Rodriguez-Salvanes F. Vancomycin MICs did not creep in *Staphylococcus aureus* isolates from 2002 to 2006 in a setting with low vancomycin usage. J Antimicrob Chemother 2008;62:773–5.
[13] Musta AC, Riederer K, Shemes S, Chase P, Jose J, Johnson LB, et al. Vancomycin MIC plus heteroresistance and outcome of methicillin-resistant *Staphylococcus aureus* bacteremia: trends over 11 years. J Clin Microbiol 2009;47:1640–4.
[14] Steinkraus G, White R, Friedrich L. Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001–05. J Antimicrob Chemother 2007;60(4):788–94.
[15] Chen SY, Hsueh PR, Chiang WC, Huang EP, Lin CF, Chang CH, et al. Predicting high vancomycin minimum inhibitory concentration isolate infection among patients with community-onset methicillin-resistant *Staphylococcus aureus* bacteraemia. J Infect 2014;69:259–65.
[16] Chen SY, Liao CH, Wang JL, Chiang WC, Lai MS, Chie WC, et al. Method-specific performance of vancomycin MIC susceptibility tests in predicting mortality of patients with methicillin-resistant *Staphylococcus aureus* bacteraemia. J Antimicrob Chemother 2014;69:211–8.
[17] Sancak B, Yagci S, Gur D, Gulay Z, Ogunc D, Soyletir G, et al. Vancomycin and daptomycin minimum inhibitory concentration distribution and occurrence of heteroresistance among methicillin-resistant *Staphylococcus aureus* blood isolates in Turkey. BMC Infect Dis 2013;13:583.
[18] Sancak B, Yagci S, Mirza HC, Hascelik G. Evaluation of vancomycin and daptomycin MIC trends for methicillin-resistant *Staphylococcus aureus* blood isolates over an 11 year period. J Antimicrob Chemother 2013;68:2689–91.
[19] Borenstein M, Higgins JP. Meta-analysis and subgroups. Prev Sci 2013;14:134–43.
[20] Cikman A, Aydin M, Gulhan B, Parlak M, Gultepe B, Kalayci Y, et al. Investigation of antibiotic resistance patterns and reduced vancomycin susceptibilities of methicillin-resistant *Staphylococcus aureus* isolates: a multi-center study. Mikrobiyol Bul 2015;49:240–8.
[21] Spagnolo AM, Orlando P, Panatto D, Amicizia D, Perdelli F, Cristina ML. *Staphylococcus aureus* with reduced susceptibility to vancomycin in health-care settings. J Prev Med Hyg 2014;55:137–44.
[22] Chang W, Ma X, Gao P, Lv X, Lu H, Chen F. Vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from 2006 to 2010 in a hospital in China. Indian J Med Microbiol 2015;33:262–6.
[23] Amatya R, Devkota P, Gautam A. Reduced susceptibility to vancomycin in methicillin resistant *Staphylococcus aureus*: a time for action. Nepal Med Coll J 2014;16:42–4.
[24] Cojutti P, Scarparo C, Sartor A, Coato P, Rigoli R, Pea FA. 5-year survey of antimicrobial susceptibility profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from patients with bloodstream infections in Northeast Italy. Diagn Microbiol Infect Dis 2015;81:53–6.
[25] Caston JJ, Gonzalez-Gasca F, Porras L, Illescas S, Romero MD, Gijon J. High vancomycin minimum inhibitory concentration is associated with poor outcome in patients with methicillin-susceptible *Staphylococcus aureus* bacteremia regardless of treatment. Scand J Infect Dis 2014;46:783–6.
[26] Casapao AM, Davis SL, McRoberts JP, Lagnf AM, Patel S, Kullar R, et al. Evaluation of vancomycin population susceptibility analysis profile as a predictor of outcomes for patients with infective endocarditis due to methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2014;58:4636–41.
[27] Panomket P, Thirat S, Wanram S, Sranujit RP. Methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to vancomycin in Sanprasitthiprasong Hospital. J Med Assoc Thai 2014;97(Suppl. 4):S7–11.
[28] Cervera C, Castaneda X, de la Maria CG, del Rio A, Moreno A, Soy D, et al. Effect of vancomycin minimal inhibitory concentration on the outcome of methicillin-susceptible *Staphylococcus aureus* endocarditis. Clin Infect Dis 2014;58:1668–75.
[29] Goldman JL, Harrison CJ, Myers AL, Jackson MA, Selvarangan R. No evidence of vancomycin minimal inhibitory concentration creep or heteroresistance identified in pediatric *Staphylococcus aureus* blood isolates. Pediatr Infect Dis J 2014;33:216–8.
[30] McDaneld PM, Spooner LM, Mohr JF, Belliveau PP. Use of daptomycin to treat infections with methicillin-resistant *Staphylococcus aureus* isolates having vancomycin minimum inhibitory concentrations of 1.5 to 2 μg/mL. Ann Pharmacother 2013;47:1654–65.
[31] Entenza JM, Betrisey B, Manuel O, Giddey M, Sakwinska O, Laurent F, et al. Rapid detection of *Staphylococcus aureus* strains with reduced susceptibility to vancomycin by isothermal microcalorimetry. J Clin Microbiol 2014;52:180–6.
[32] Wang JL, Lai CH, Lin HH, Chen WF, Shih YC, Hung CH. High vancomycin minimum inhibitory concentrations with heteroresistant vancomycin-intermediate *Staphylococcus aureus* in methicillin-resistant *S. aureus* bacteraemia patients. Int J Antimicrob Agents 2013;42:390–4.
[33] Park SY, Oh IH, Lee HJ, Ihm CG, Son JS, Lee MS, et al. Impact of reduced vancomycin MIC on clinical outcomes of methicillin-resistant *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother 2013;57:5536–42.
[34] Yang W, He B, Ning YZ, Li Y. The change and significance of vancomycin minimal inhibitory concentration against methicillin-resistant *Staphylococcus aureus* isolates from inpatients with lower respiratory tract infection. Zhonghua Jie He He Hu Xi Za Zhi 2013;36:288–92.
[35] Lepe JA, Dominguez-Herrera J, Pachon J, Aznar J. Determining accurate vancomycin MIC values for methicillin-resistant *Staphylococcus aureus* by the microdilution method. J Antimicrob Chemother 2014;69:136–8.
[36] Guzek A, Korzeniewski K, Nitsch-Osuch A, Rybicki Z, Prokop E. In vitro susceptibility of Staphylococci and Enterococci to vancomycin and teicoplanin. Adv Exp Med Biol 2013;788:125–32.
[37] Hope R, Blackburn RM, Verlander NQ, Johnson AP, Kearns A, Hill R, et al. Vancomycin MIC as a predictor of outcome in MRSA bacteraemia in the UK context. J Antimicrob Chemother 2013;68:2641–7.
[38] Huang CH, Chen YH. The detection and clinical impact of vancomycin MIC among patients with methicillin-resistant *Staphylococcus aureus* bacteremia. J Microbiol Immunol Infect 2013;46:315–6.
[39] Kizilarslanoglu MC, Sancak B, Yagci S, Hascelik G, Unal S. Evaluation of methicillin-resistant *Staphylococcus aureus* bacteremia and comparison of prognosis according to vancomycin MIC values: experience of the last ten years. Mikrobiyol Bul 2013;47:199–210.
[40] Zhuo C, Xu YC, Xiao SN, Zhang GY, Zhong NS. Glycopeptide minimum inhibitory concentration creep among meticillin-resistant *Staphylococcus aureus* from 2006–2011 in China. Int J Antimicrob Agents 2013;41:578–81.
[41] Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MV, et al. Vancomycin minimum inhibitory concentration, host comorbidities and mortality in *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect 2013;19:1163–8.

Please cite this article in press as: Diaz R, et al., Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections—a systematic review and meta-analysis, Clinical Microbiology and Infection (2017), http://dx.doi.org/10.1016/j.cmi.2017.06.017

[42] Joana S, Pedro P, Elsa G, Filomena M. Is vancomycin MIC creep a worldwide phenomenon? Assessment of *S. aureus* vancomycin MIC in a tertiary university hospital. BMC Res Notes 2013;6:65.
[43] Khatib R, Riederer K, Shemes S, Musta AC, Szpunar S. Correlation of methicillin-resistant *Staphylococcus aureus* vancomycin minimal inhibitory concentration results by Etest and broth microdilution methods with population analysis profile: lack of Etest overestimation of the MIC. Eur J Clin Microbiol Infect Dis 2013;32:803–6.
[44] Abdelhady W, Bayer AS, Seidl K, Nast CC, Kiedrowski MR, Horswill AR, et al. Reduced vancomycin susceptibility in an in vitro catheter-related biofilm model correlates with poor therapeutic outcomes in experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2013;57:1447–54.
[45] Woods CJ, Chowdhury A, Patel VM, Shorr AF. Impact of vancomycin minimum inhibitory concentration on mortality among critically ill patients with methicillin-resistant *Staphylococcus aureus* bacteremia. Infect Control Hosp Epidemiol 2012;33:1246–9.
[46] Jacob JT, DiazGranados CA. High vancomycin minimum inhibitory concentration and clinical outcomes in adults with methicillin-resistant *Staphylococcus aureus* infections: a meta-analysis. Int J Infect Dis 2013;17:e93–100.
[47] Reynolds R, Hope R, Warner M, MacGowan AP, Livermore DM, Ellington MJ. Lack of upward creep of glycopeptide MICs for methicillin-resistant *Staphylococcus aureus* (MRSA) isolated in the UK and Ireland 2001-07. J Antimicrob Chemother 2012;67:2912–8.
[48] Vaudaux P, Ferry T, Uckay I, Francois P, Schrenzel J, Harbarth S, et al. Prevalence of isolates with reduced glycopeptide susceptibility in orthopedic device-related infections due to methicillin-resistant *Staphylococcus aureus*. Eur J Clin Microbiol Infect Dis 2012;31:3367–74.
[49] Han JH, Mascitti KB, Edelstein PH, Bilker WB, Lautenbach E. Effect of reduced vancomycin susceptibility on clinical and economic outcomes in *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother 2012;56:5164–70.
[50] Han JH, Edelstein PH, Lautenbach E. Reduced vancomycin susceptibility and staphylococcal cassette chromosome mec (SCCmec) type distribution in methicillin-resistant *Staphylococcus aureus* bacteraemia. J Antimicrob Chemother 2012;67:2346–9.
[51] Wi YM, Kim JM, Joo EJ, Ha YE, Kang CI, Ko KS, et al. High vancomycin minimum inhibitory concentration is a predictor of mortality in meticillin-resistant *Staphylococcus aureus* bacteraemia. Int J Antimicrob Agents 2012;40:108–13.
[52] Chong YP, Park SJ, Kim HS, Kim ES, Kim MN, Kim SH, et al. In vitro activities of ceftobiprole, dalbavancin, daptomycin, linezolid, and tigecycline against methicillin-resistant *Staphylococcus aureus* blood isolates: stratified analysis by vancomycin MIC. Diagn Microbiol Infect Dis 2012;73:264–6.
[53] Calfee DP. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci, and other Gram-positives in healthcare. Curr Opin Infect Dis 2012;25:385–94.
[54] Holmes NE, Johnson PD, Howden BP. Relationship between vancomycin-resistant *Staphylococcus aureus*, vancomycin-intermediate *S. aureus*, high vancomycin MIC, and outcome in serious *S. aureus* infections. J Clin Microbiol 2012;50:2548–52.
[55] Rojas L, Bunsow E, Munoz P, Cercenado E, Rodriguez-Creixems M, Bouza E. Vancomycin MICs do not predict the outcome of methicillin-resistant *Staphylococcus aureus* bloodstream infections in correctly treated patients. J Antimicrob Chemother 2012;67:1760–8.
[56] Zhao C, Sun H, Wang H, Liu Y, Hu B, Yu Y, et al. Antimicrobial resistance trends among 5608 clinical Gram-positive isolates in China: results from the Gram-Positive Cocci Resistance Surveillance program (2005–2010). Diagn Microbiol Infect Dis 2012;73:174–81.
[57] van Hal SJ, Lodise TP, Paterson DL. The clinical significance of vancomycin minimum inhibitory concentration in *Staphylococcus aureus* infections: a systematic review and meta-analysis. Clin Infect Dis 2012;54:755–71.
[58] Thati V, Shivannavar CT, Gaddad SM. Vancomycin resistance among methicillin resistant *Staphylococcus aureus* isolates from intensive care units of tertiary care hospitals in Hyderabad. Indian J Med Res 2011;134:704–8.
[59] Chang HJ, Hsu PC, Yang CC, Siu LK, Kuo AJ, Chia JH, et al. Influence of teicoplanin MICs on treatment outcomes among patients with teicoplanin-treated methicillin-resistant *Staphylococcus aureus* bacteraemia: a hospital-based retrospective study. J Antimicrob Chemother 2012;67:736–41.
[60] Honda H, Doern CD, Michael-Dunne Jr W, Warren DK. The impact of vancomycin susceptibility on treatment outcomes among patients with methicillin resistant *Staphylococcus aureus* bacteremia. BMC Infect Dis 2011;11:335.
[61] Pelitli TS, Cesur S, Kinikli S, Irmak H, Demiroz AP, Karakoc E. Evaluation of vancomycin, teicoplanin, linezolide and tigecycline susceptibilities of nosocomial methicillin-resistant *Staphylococcus* strains by E-test. Mikrobiyol Bul 2011;45:758–61.
[62] Clemens EC, Chan JD, Lynch JB, Dellit TH. Relationships between vancomycin minimum inhibitory concentration, dosing strategies, and outcomes in methicillin-resistant *Staphylococcus aureus* bacteremia. Diagn Microbiol Infect Dis 2011;71:408–14.
[63] de Sanctis JT, Swami A, Sawarynski K, Gerasymchuk L, Powell K, Robinson-Dunn B, et al. Is there a clinical association of vancomycin MIC creep, agr group II locus, and treatment failure in MRSA bacteremia? Diagn Mol Pathol 2011;20:184–8.
[64] Kuscu F, Ozturk DB, Gurbuz Y, Tutuncu EE, Sencan I, Gul S. Investigation of reduced vancomycin susceptibility in methicillin-resistant staphylococci. Mikrobiyol Bul 2011;45:248–57.
[65] Kao TM, Wang JT, Weng CM, Chen YC, Chang SC. In vitro activity of linezolid, tigecycline, and daptomycin on methicillin-resistant *Staphylococcus aureus* blood isolates from adult patients, 2006–2008: stratified analysis by vancomycin MIC. J Microbiol Immunol Infect 2011;44:346–51.
[66] Laible BR, Hellwig TR, Hedge DD. Susceptibility of *Staphylococcus aureus* to vancomycin: analysis of minimum inhibitory concentrations in two tertiary care hospitals in eastern South Dakota. S D Med 2011;64:91–5.
[67] Lubin AS, Snydman DR, Ruthazer R, Bide P, Golan Y. Predicting high vancomycin minimum inhibitory concentration in methicillin-resistant *Staphylococcus aureus* bloodstream infections. Clin Infect Dis 2011;52:997–1002.
[68] Choi EY, Huh JW, Lim CM, Koh Y, Kim SH, Choi SH, et al. Relationship between the MIC of vancomycin and clinical outcome in patients with MRSA nosocomial pneumonia. Intensive Care Med 2011;37:639–47.
[69] Traverso F, Peluffo M, Louge M, Funaro F, Suasnabar R, Cepeda R. Impact of methicillin resistance on mortality and surveillance of vancomycin susceptibility in bacteremias caused by *Staphylococcus aureus*. Rev Argent Microbiol 2010;42:274–8.
[70] Bukhari SZ, Ahmed S, Zia N. Antimicrobial susceptibility pattern of *Staphylococcus aureus* on clinical isolates and efficacy of laboratory tests to diagnose MRSA: a multi-centre study. J Ayub Med Coll Abbottabad 2011;23:139–42.
[71] Keel RA, Sutherland CA, Aslanzadeh J, Nicolau DP, Kuti JL. Correlation between vancomycin and daptomycin MIC values for methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* by 3 testing methodologies. Diagn Microbiol Infect Dis 2010;68:326–9.
[72] Bland CM, Porr WH, Davis KA, Mansell KB. Vancomycin MIC susceptibility testing of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* isolates: a comparison between Etest® and an automated testing method. South Med J 2010;103:1124–8.
[73] Machado DP, Nagel F, Aquino VR, de Souza Martins D, Nazario R, Goldani LZ, et al. Vancomycin minimal inhibitory concentration from broth microdilution and Etest in respiratory tract samples of patients with ventilation-associated pneumonia. J Hosp Infect 2010;76:182–4.
[74] Haque NZ, Zuniga LC, Peyrani P, Reyes K, Lamerato L, Moore CL, et al. Relationship of vancomycin minimum inhibitory concentration to mortality in patients with methicillin-resistant *Staphylococcus aureus* hospital-acquired, ventilator-associated, or health-care-associated pneumonia. Chest 2010;138:1356–62.
[75] Aktas E, Mengeloglu FZ, Kulah C, Comert FB. Evaluation of reduced susceptibility to vancomycin among MRSA strains isolated from clinical specimens. Mikrobiyol Bul 2010;44:339–41.
[76] Wang JL, Wang JT, Sheng WH, Chen YC, Chang SC. Nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia in Taiwan: mortality analyses and the impact of vancomycin, MIC = 2 mg/L, by the broth microdilution method. BMC Infect Dis 2010;10:159.
[77] Zheng X, Qi C, Arrieta M, O'Leary A, Wang D, Shulman ST. Lack of increase in vancomycin resistance of pediatric methicillin-resistant *Staphylococcus aureus* isolates from 2000 to 2007. Pediatr Infect Dis J 2010;29:882–4.
[78] Tiwari HK, Das AK, Sapkota D, Sivrajan K, Pahwa VK. Methicillin resistant *Staphylococcus aureus*: prevalence and antibiogram in a tertiary care hospital in western Nepal. J Infect Dev Ctries 2009;3:681–4.
[79] Patel N, Lubanski P, Ferro S, Bonafede M, Harrington S, Evans A, et al. Correlation between vancomycin MIC values and those of other agents against gram-positive bacteria among patients with bloodstream infections caused by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2009;53:5141–4.
[80] Pillai SK, Wennersten C, Venkataraman L, Eliopoulos GM, Moellering RC, Karchmer AW. Development of reduced vancomycin susceptibility in methicillin-susceptible *Staphylococcus aureus*. Clin Infect Dis 2009;49:1169–74.
[81] Kuti JL, Nicasio AM, Sutherland CA, Nicolau DP. Elevated vancomycin minimum inhibitory concentrations among methicillin-resistant *Staphylococcus aureus* isolated from patients with ventilator-associated pneumonia at a Connecticut hospital. Conn Med 2009;73:337–40.
[82] Marais E, Aithma N, Perovic O, Oosthuysen WF, Musenge E, Duse AG. Antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolates from South Africa. S Afr Med J 2009;99:170–3.
[83] Horne KC, Howden BP, Grabsch EA, Graham M, Ward PB, Xie S, et al. Prospective comparison of the clinical impacts of heterogeneous vancomycin-intermediate methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-susceptible MRSA. Antimicrob Agents Chemother 2009;53:3447–52.
[84] Sader HS, Rhomberg PR, Jones RN. Nine-hospital study comparing broth microdilution and Etest method results for vancomycin and daptomycin against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2009;53:3162–5.
[85] Beeston CJ, Gupta R, Chadwick PR, Young RJ. Methicillin-resistant *Staphylococcus aureus* bacteraemia and mortality in a teaching hospital. Eur J Clin Microbiol Infect Dis 2009;28:585–90.
[86] Lodise TP, Miller CD, Graves J, Evans A, Graffunder E, Helmecke M, et al. Predictors of high vancomycin MIC values among patients with methicillin-resistant *Staphylococcus aureus* bacteraemia. J Antimicrob Chemother 2008;62:1138–41.

Please cite this article in press as: Diaz R, et al., Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections—a systematic review and meta-analysis, Clinical Microbiology and Infection (2017), http://dx.doi.org/10.1016/j.cmi.2017.06.017

[87] Lodise TP, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro BM, et al. Relationship between vancomycin MIC and failure among patients with methicillin-resistant *Staphylococcus aureus* bacteremia treated with vancomycin. Antimicrob Agents Chemother 2008;52:3315–20.
[88] Hosgor Limoncu M, Ermertcan S, Tasli H, Kurutepe S. Investigation of glycopeptide resistance in methicillin resistant staphylococcal isolates. Mikrobiyol Bul 2007;41:511–6.
[89] Bennett JW, Murray CK, Holmes RL, Patterson JE, Jorgensen JH. Diminished vancomycin and daptomycin susceptibility during prolonged bacteremia with methicillin-resistant *Staphylococcus aureus*. Diagn Microbiol Infect Dis 2008;60:437–40.
[90] Chi CY, Lauderdale TL, Wang SM, Wu JM, Yang YJ, Liu CC. Health care-associated endocarditis caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin. J Clin Microbiol 2008;46:810–3.
[91] Lewis 2nd JS, Ellis MW. Approaches to serious methicillin-resistant *Staphylococcus aureus* infections with decreased susceptibility to vancomycin: clinical significance and options for management. Curr Opin Infect Dis 2007;20:568–73.
[92] Neoh HM, Hori S, Komatsu M, Oguri T, Takeuchi F, Cui L, et al. Impact of reduced vancomycin susceptibility on the therapeutic outcome of MRSA bloodstream infections. Ann Clin Microbiol Antimicrob 2007;6:13.
[93] Appelbaum PC. Reduced glycopeptide susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA). Int J Antimicrob Agents 2007;30:398–408.
[94] Fitzgibbon MM, Rossney AS, O'Connell B. Investigation of reduced susceptibility to glycopeptides among methicillin-resistant *Staphylococcus aureus* isolates from patients in Ireland and evaluation of agar screening methods for detection of heterogeneously glycopeptide-intermediate *S. aureus*. J Clin Microbiol 2007;45:3263–9.
[95] Graber CJ, Wong MK, Carleton HA, Perdreau-Remington F, Haller BL, Chambers HF. Intermediate vancomycin susceptibility in a community-associated MRSA clone. Emerg Infect Dis 2007;13:491–3.
[96] Biedenbach DJ, Bell JM, Sader HS, Fritsche TR, Jones RN, Turnidge JD. Antimicrobial susceptibility of Gram-positive bacterial isolates from the Asia-Pacific region and an in vitro evaluation of the bactericidal activity of daptomycin, vancomycin, and teicoplanin: a SENTRY Program Report (2003–2004). Int J Antimicrob Agents 2007;30:143–9.
[97] Murata T, Otani K. Nosocomial infection caused by multidrug-resistant *Staphylococcus aureus* with reduced susceptibility to vancomycin and teicoplanin. Yamagata Prefecture, Japan; May 2004–Jun 2005. Kansenshogaku Zasshi 2007;81:183–8.
[98] Wang G, Hindler JF, Ward KW, Bruckner DA. Increased vancomycin MICs for *Staphylococcus aureus* clinical isolates from a university hospital during a 5-year period. J Clin Microbiol 2006;44:3883–6.
[99] Webster D, Rennie RP, Brosnikoff CL, Chui L, Brown C. Methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to vancomycin in Canada. Diagn Microbiol Infect Dis 2007;57:177–81.
[100] Bhat G, Kamath S, Hussain A. Nosocomial methicillin–resistant *Staphylococcus aureus* with reduced susceptibility to vancomycin. Indian J Pathol Microbiol 2006;49:311–2.
[101] Baddour MM, Abuelkheir MM, Fatani AJ. Trends in antibiotic susceptibility patterns and epidemiology of MRSA isolates from several hospitals in Riyadh, Saudi Arabia. Ann Clin Microbiol Antimicrob 2006;5:30.
[102] Samra Z, Ofer O, Shmuely H. Susceptibility of methicillin-resistant *Staphylococcus aureus* to vancomycin, teicoplanin, linezolid, pristinamycin and other antibiotics. Isr Med Assoc J 2005;7:148–50.
[103] Nishijima S, Kurokawa I, Nakaya H. Susceptibility change to antibiotics of *Staphylococcus aureus* strains isolated from skin infections between July 1994 and November 2000. J Infect Chemother 2002;8:187–9.
[104] Mallaval FO, Carricajo A, Delavenna F, Recule C, Fonsale N, Manquat G, et al. Detection of an outbreak of methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to glycopeptides in a French hospital. Clin Microbiol Infect 2004;10:459–61.
[105] Clinical and Laboratory Standards Institute C. Performance standards for antimicrobial susceptibility testing. In: Twenty-fourth informational Supplement. Wayne, PA: CLSI; 2014.
[106] Jones RN. Microbiological features of vancomycin in the 21st century: minimum inhibitory concentration creep, bactericidal/static activity, and applied breakpoints to predict clinical outcomes or detect resistant strains. Clin Infect Dis 2006;1:S13–24.
[107] Sader HS, Fey PD, Limaye AP, Madinger N, Pankey G, Rahal J, et al. Evaluation of vancomycin and daptomycin potency trends (MIC creep) against methicillin-resistant *Staphylococcus aureus* isolates collected in nine U.S. medical centers from 2002 to 2006. Antimicrob Agents Chemother 2009;53:4127–32.

Please cite this article in press as: Diaz R, et al., Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections—a systematic review and meta-analysis, Clinical Microbiology and Infection (2017), http://dx.doi.org/10.1016/j.cmi.2017.06.017

## Article **"Evaluation of vancomycin MIC creep in *Staphylococcus aureus*"**

Raquel Diaz, Elmano Ramalheira, Vera Afreixo, Bruno Gago
Journal of Global Antimicrobial Resistance 10 (2017) 281–284
URL: http://www.jgaronline.com/article/S2213-7165(17)30088-7/fulltext
DOI: 10.1016/j.jgar.2017.04.007
PMID: 28751240

Journal of Global Antimicrobial Resistance 10 (2017) 281–284

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Short Communication

# Evaluation of vancomycin MIC creep in *Staphylococcus aureus*

Raquel Diaz[a,b,c,*], Elmano Ramalheira[a,c], Vera Afreixo[a,b,d], Bruno Gago[a,b]

[a] Health Sciences Department, University of Aveiro, 3810-193 Aveiro, Portugal
[b] Institute for Research in Biomedicine (iBiMED), Health Sciences Program, University of Aveiro, 3810-193 Aveiro, Portugal
[c] Department of Medical Microbiology, Centro Hospitalar Baixo Vouga, Av. Artur Ravara, 3814-501 Aveiro, Portugal
[d] Department of Mathematics, University of Aveiro, 3810-193 Aveiro, Portugal

ARTICLE INFO

*Article history:*
Received 8 October 2016
Received in revised form 3 April 2017
Accepted 5 April 2017
Available online 25 July 2017

*Keywords:*
*Staphylococcus aureus*
Vancomycin
MIC creep
Susceptibility testing methods

ABSTRACT

*Objectives:* Vancomycin is the primary treatment for methicillin-resistant *Staphylococcus aureus* (MRSA). However, an increasing proportion of MRSA isolates with high minimum inhibitory concentrations (MICs) within the susceptible range (vancomycin 'MIC creep') is being observed. The aim of this study was to assess the vancomycin MIC distribution for *S. aureus* isolates over a period of 4 years in Centro Hospitalar Baixo Vouga (Aveiro, Portugal) and to identify differences in vancomycin MIC determined by different susceptibility testing methods.
*Methods:* For each *S. aureus* isolate, the vancomycin MIC was assayed by the VITEK®2 automated system and the broth microdilution testing method.
*Results:* The results showed significant differences in vancomycin MIC by different methods ($P = 0.021$, sign test) and did not suggest the presence of vancomycin MIC creep during the study period.
*Conclusions:* Vancomycin MIC creep is a regional problem, therefore it can only be assessed through the evaluation of local susceptibility profiles, and antibiogram based on real MIC assay should be an essential element in local MRSA infection clinical management.


## 1. Introduction

*Staphylococcus aureus* is one of the most common pathogens causing severe infections both in community and hospital settings. Generally these infections are associated with increased morbidity, longer antibiotic therapy, higher healthcare costs, prolonged hospitalisation and increased risk of death [1]. The first antibiotic agent used for the treatment of invasive methicillin-resistant *S. aureus* (MRSA) infections is the glycopeptide vancomycin, which remains the gold-standard approach [2]. Utilisation of vancomycin has been increasing since the mid-1980s, resulting in the emergence of MRSA with reduced vancomycin susceptibility [1]. Recently, a phenomenon of gradual increase in glycopeptide minimum inhibitory concentrations (MICs) for *S. aureus* has been observed and is described in the literature as 'MIC creep' [3].

Patients with isolates exhibiting MIC creep have been associated with poor clinical outcomes, including delayed response, increased mortality, increased rate of relapse, prolonged hospital stay and overall increased cost of hospitalisation [1,4,5]. Publications regarding MIC creep include studies that report an increase in the mean vancomycin MIC as well as studies that do not confirm these findings in MRSA [2].

Combining data from multiple centres can obscure trends that may exist within a given institution(s) or country as a result of differences in patient populations and drug usage patterns. Therefore, it is important to study vancomycin MIC creep as a regional problem, and evaluation of local susceptibility profiles should be essential in local MRSA infection clinical management [6]. Owing to evidence of MIC creep in some regions, the aim of this study was to assess the vancomycin MIC distribution for *S. aureus* isolates over a period of 4 years in Centro Hospitalar Baixo Vouga (CHBV) (Aveiro, Portugal). The study aimed to identify trends and differences in vancomycin MICs determined by different susceptibility testing methods. Detection of a tendency for vancomycin MIC creep in MRSA strains in a particular population should prompt regional health authorities to include these data in future discussions regarding treatment protocols and strategies.

* Corresponding author at: Health Sciences Department, University of Aveiro, 3810-193 Aveiro, Portugal.
*E-mail address:* raqueldiaz@ua.pt (R. Diaz).

http://dx.doi.org/10.1016/j.jgar.2017.04.007

## 2. Materials and methods

### 2.1. Aims and study design

This study aimed to assess the possibility of vancomycin MIC creep amongst *S. aureus* isolates from various clinical sources. Second, it aimed to identify differences in vancomycin MIC determined by different susceptibility testing methods. Vancomycin susceptibility was comparatively evaluated by two testing methods, namely the VITEK®2 automated system (bioMérieux, France) [7] and the broth microdilution (BMD) method [8].

### 2.2. Study population and data collection

A total of 101 clinical *S. aureus* isolates obtained between 2010 and 2013 from CHBV were analysed. Isolates were identified by routine bacteriological procedures. Only one isolate per patient was included. Isolates were collected from various clinical sources, including respiratory tract ($n = 36$), blood ($n = 22$), wounds ($n = 38$), urine ($n = 2$) and others ($n = 3$). These isolates were included in the isolates that were sent to the SENTRY antimicrobial surveillance programme. Patient demographic characteristics and clinical data were obtained from the medical records. From the 101 isolates, only 95 had data available for the two methodologies.

### 2.3. Laboratory methods

For each *S. aureus* isolate, the vancomycin MIC was assayed for methicillin-susceptible *S. aureus* (MSSA) and MRSA by the two testing methods. The susceptible breakpoint for vancomycin was 2 mg/L [9]. Susceptibility testing was performed using the VITEK®2 instrument with commercially available *Staphylococcus* cards (GP, AST619; bioMérieux) and the same isolates were directed to SENTRY for the evaluation of vancomycin MICs by the BMD method.

### 2.4. Statistical analysis

Vancomycin MICs were dichotomised (MIC ≤ 0.5 mg/L and MIC = 1 mg/L). The association between categorical variables was determined by $\chi^2$ test. Quantitative variables were compared using the non-parametric paired-sign test, Kruskal–Wallis test or Mann–Whitney test. The $\chi^2$ test was used for MIC trends over time. Comparison between the two methods was performed by means of the Spearman's non-parametric correlation.

All statistical analyses were performed using IBM SPSS Statistics v.22.0 (IBM Corp., Armonk, NY), and tests with a *P*-value of $<0.05$ were considered statistically significant.

## 3. Results

A total of 101 *S. aureus* isolates were analysed during the study period. The majority of isolates were collected from the respiratory tract and wounds. The studied groups were homogeneous and comparable. The demographic and clinical characteristics of the patients are summarised in Table 1. The main cause of infection was MRSA, the leading cause of hospitalisation was tract respiratory disease, and the two main co-morbidities were arterial hypertension and diabetes.

Table 2 shows a comparison of the clinical presentation data and outcomes between the vancomycin MIC ≤ 0.5 mg/L and MIC = 1 mg/L groups. No significant differences were found in in-

**Table 1**
Demographic characteristics, clinical presentation and clinical outcomes of 101 patients from Centro Hospitalar Baixo Vouga (Aveiro, Portugal) with *Staphylococcus aureus* infections between 2010–2013.

| | 2010 ($n = 23$) | 2011 ($n = 31$) | 2012 ($n = 24$) | 2013 ($n = 23$) | *P*-value |
|---|---|---|---|---|---|
| Age (years) (mean ± S.D.) | 73 ± 18 | 70 ± 19 | 73 ± 16 | 72 ± 10 | 0.676 |
| Male sex [*n* (%)] | 13 (56.5) | 18 (58.1) | 15 (62.5) | 14 (60.9) | 0.975 |
| Cause of hospitalisation (*n*) | | | | | |
| Anaemia | 0 | 1 | 0 | 0 | 0.516 |
| Diabetes | 1 | 1 | 0 | 0 | 0.602 |
| Cardiovascular disease | 3 | 5 | 0 | 5 | 0.142 |
| Respiratory disease | 13 | 6 | 10 | 8 | 0.182 |
| Genitourinary disease | 2 | 2 | 2 | 0 | 0.567 |
| Gastrointestinal tract disease | 0 | 4 | 4 | 2 | 0.250 |
| Systemic infection | 3 | 0 | 2 | 1 | 0.223 |
| Osteoarticular infection | 1 | 4 | 2 | 1 | 0.601 |
| Central nervous system infection | 0 | 1 | 0 | 1 | 0.602 |
| Neurological disease | 0 | 3 | 0 | 1 | 0.203 |
| Other | 0 | 4 | 4 | 4 | – |
| Co-morbidities (*n*) | | | | | |
| Diabetes | 8 | 10 | 8 | 7 | 0.991 |
| Cancer | 2 | 1 | 1 | 1 | 0.818 |
| Anaemia | 1 | 5 | 1 | 1 | 0.247 |
| Heart disease | 4 | 7 | 4 | 8 | 0.428 |
| Alcoholism | 0 | 1 | 3 | 0 | 0.088 |
| Neurological disease | 7 | 4 | 5 | 5 | 0.478 |
| Kidney failure | 7 | 6 | 9 | 2 | 0.099 |
| Respiratory disease | 3 | 6 | 4 | 0 | 0.182 |
| Hepatitis | 0 | 1 | 0 | 0 | 0.516 |
| Arterial hypertension | 10 | 9 | 9 | 11 | 0.519 |
| Osteoarticular system disease | 1 | 0 | 2 | 1 | 0.475 |
| Skin and soft-tissue infection | 9 | 1 | 2 | 1 | 0.000 |
| Vancomycin BMD MIC ≤ 0.5 mg/L (*n*) | 14 | 13 | 12 | 12 | 0.403 |
| In-hospital mortality (*n*) | 7 | 3 | 6 | 6 | 0.254 |
| MRSA (*n*)[a] | 19 | 26 | 22 | 21 | 0.68 |
| MSSA (*n*)[a] | 4 | 6 | 3 | 2 | 0.704 |

S.D., standard deviation; BMD, broth microdilution; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.
[a] Two patients had both MRSA and MSSA isolates.

**Table 2**
Comparison of clinical presentation data and outcomes between vancomycin MIC ≤ 0.5 mg/L and MIC = 1.0 mg/L groups by the broth microdilution (BMD) method ($n$ = 95).

| | MIC ≤ 0.5 mg/L | MIC = 1.0 mg/L | *P*-value |
|---|---|---|---|
| No. of patients | 51 | 44 | |
| Age (years) (mean ± S.D.) | 72 ± 14 | 70 ± 18 | 0.964 |
| Male sex ($n$) | 32 | 24 | 0.418 |
| | | | |
| Cause of hospitalisation ($n$) | | | |
| Anaemia | 0 | 1 | 0.279 |
| Diabetes | 2 | 0 | 0.184 |
| Cardiovascular disease | 4 | 9 | 0.075 |
| Respiratory disease | 21 | 13 | 0.238 |
| Genitourinary disease | 5 | 1 | 0.132 |
| Gastrointestinal tract disease | 6 | 4 | 0.672 |
| Systemic infection | 5 | 1 | 0.132 |
| Osteoarticular infection | 3 | 5 | 0.337 |
| Central nervous system infection | 1 | 1 | 0.916 |
| Neurological disease | 3 | 1 | 0.382 |
| Other | 1 | 8 | |
| | | | |
| Co-morbidities ($n$) | | | |
| Diabetes | 17 | 12 | 0.522 |
| Cancer | 4 | 1 | 0.225 |
| Anaemia | 4 | 4 | 0.827 |
| Heart disease | 11 | 11 | 0.693 |
| Alcoholism | 0 | 3 | 0.058 |
| Neurological disease | 10 | 11 | 0.528 |
| Kidney failure | 15 | 5 | 0.031 |
| Respiratory disease | 9 | 4 | 0.226 |
| Hepatitis | 0 | 1 | 0.279 |
| Arterial hypertension | 22 | 15 | 0.367 |
| Osteoarticular system disease | 2 | 1 | 0.647 |
| Skin and soft-tissue infection | 2 | 1 | 0.990 |
| In-hospital mortality ($n$) | 13 | 9 | 0.562 |
| MRSA ($n$)[a] | 47 | 35 | 0.075 |
| MSSA ($n$)[a] | 4 | 11 | 0.022 |

MIC, minimum inhibitory concentration; S.D., standard deviation; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.
[a] Two patients had both MRSA and MSSA isolates.

hospital mortality rate ($P$ = 0.562) amongst patients harbouring isolates with isolates with different MICs. Infection with MSSA was strongly associated ($P$ = 0.022) with higher MICs (1 mg/L).

All of the studied *S. aureus* isolates were susceptible to vancomycin, exhibiting MICs below the current Clinical and Laboratory Standards Institute (CLSI) breakpoint (<2 mg/L) [9].

A significant association between MICs using the two methods and the year ($P$ = 0.006, $\chi^2$ test) was obtained. In addition, evaluation of vancomycin MICs by method showed significant differences for the same isolate ($P$ = 0.021, sign test).

Correlations between MIC methods results and year were evaluated. Spearman's correlation coefficient between year and BMD was −0.006 ($P$ = 0.957) and between year and automated system analysis was 0.360 ($P$ = 0.000), supporting the results in Fig. 1.

Automated system vancomycin MICs were also significantly lower (71%, MIC ≤ 0.5 mg/L; 29% MIC 1 = mg/L) than those found by BMD analysis (54%, MIC ≤ 0.5 mg/L; 46% MIC = 1 mg/L) ($P$ = 0.007, sign test).

## 4. Discussion

Vancomycin MIC creep in *S. aureus* is a phenomenon whereby isolates with higher vancomycin MICs are becoming more common, although MICs remain within the susceptible range of the drug [10]. Studies reporting changes over time in MRSA isolates have revealed conflicting results, with MIC creep noted in some populations but not in others [1]. This study aimed to analyse trends in vancomycin MICs obtained using an automated system and BMD between 2010 and 2013 for *S. aureus* isolates collected

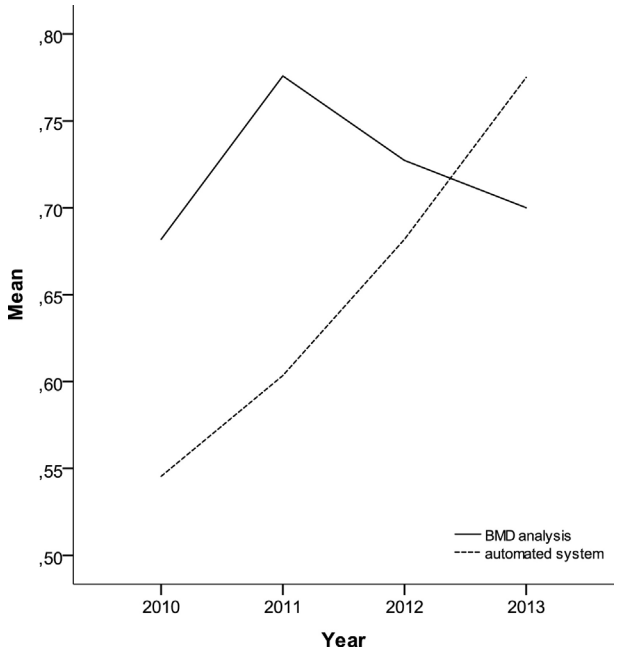


**Fig. 1.** Mean vancomycin minimum inhibitory concentrations (MICs) between 2010 and 2013 analysed by the VITEK®2 automated system (---) and the broth microdilution (BMD) method (—).

from various clinical sources. For BMD analysis, no significant increase was found, a result consistent with others studies, e.g. the SENTRY database in which between 1998–2003 a total of 35 458 *S. aureus* isolates were studied [11], a multicentre study of nine US medical centres with 1800 MRSA samples between 1999–2006 [12] and a survey from Spain between 2002–2006 with 3141 *S. aureus* isolates [13].

On the other hand, increasing vancomycin MICs were reported in a single centre in France that studied 1445 MRSA between 1983–2002 [1,13], a multiple single-centre study in the USA that studied 662 MRSA between 1999–2006 [14,15] and a study in Hong Kong that analysed 247 MRSA between 1997–2008 [16].

Noteworthy, in a study that examined 287 MRSA isolates from blood cultures collected at several hospitals in two German cities between 2004–2009, an increase in vancomycin MIC was detected in only one city, suggesting a regional problem and highlighting the importance of regional studies as well as the need to empower regional health centres to monitor their local status [2].

Nevertheless, the reason for the detection of the MIC creep phenomenon in some centres but not in all has not yet been completely elucidated. The high heterogeneity of the studies make a direct comparison difficult. Indeed, studies differ in the analysed period, type of isolates, clinical source of the isolates as well the methods used for susceptibility testing [2,5]. The reports reveal that there are studies which did not find vancomycin MIC creep using the BMD method [17] or Etest [18] and others that found changes using the same methodologies [14,15,17].

Reports suggest that the Etest method is more sensitive in detecting small vancomycin MIC changes than the BMD and the automated system [1,2]. The reliability of the automated method applied to measure the MIC of glycopeptides for *S. aureus* must be treated with caution. In the results of this work, considerable differences between methods were observed. Automated analysis shows some tendency for MIC creep, whilst in BMD the gradual increase in MIC does not show any tendency. The automated system provided MICs of vancomycin ≤0.5 mg/L in 71% of cases and 1 mg/L in 29% of cases. On the other hand, with the BMD an MIC of 0.5 mg/L was obtained in 54% of cases and 1 mg/L in 46% of cases.

These results show that vancomycin MICs from the automated method were also significantly lower than those by BMD. Owing to these significant differences in the results obtained by both methods as well as according to reports in the literature showing that there is a higher probability of clinical success when the MIC of vancomycin does not exceed a value of 1.0 mg/L, it is important to underline the fact that the real MIC assayed by means of Etest or BMD is more useful than that provided by the automated method [3,19]. These findings are consistent with the results from the study by Tomczak et al. that compared the MIC obtained with the automated method and Etest for *S. aureus* strains [3].

The results presented here have some limitations. First, this was a retrospective single-centre study with possible sample selection bias. In addition, longer periods are needed for a broad statement about trends. Nevertheless, this is the first study in Aveiro and the second in Portugal, giving relevance to this study. Silvestre et al. evaluated the presence of vancomycin MIC creep in 93 MRSA from 2007–2009 in a hospital in Lisbon (Portugal) and did not find data compatible with the presence of vancomycin MIC creep [20].

Since this appears to be a regional problem, we suggest that all hospitals should monitor their local status of vancomycin MICs in order to evaluate the presence of trends and to ensure the effectiveness of vancomycin therapy. Considering our results, it is important in our hospital to evaluate vancomycin MICs during the next years in order to observe whether differences between two methodologies continue and whether the automated method continues to shows some tendency for MIC creep.

## 5. Conclusion

This study showed significant differences in vancomycin MIC with different methods and no evidence of vancomycin MIC creep. The relevance of vancomycin creep for individual clinicians, pharmacists and microbiologists can only be assessed through the evaluation of local susceptibility profiles and not from published observations of other researchers. The antibiogram based on the real MIC assay should be an essential element when vancomycin therapy is instituted in the clinical management of MRSA infections, avoiding whenever possible the use of vancomycin MIC based on automated methods.

## Funding

This work was supported by Portuguese funds through the Institute of Biomedicine (iBiMED), the Institute of Electronics and Telematics Engineering of Aveiro (IEETA) and the Portuguese Foundation for Science and Technology (FCT–Fundação para a Ciência e Tecnologia), within project COMPETE/FEDER UID/BIM/04501/2013. Vancomycin MIC data were generously provided by the SENTRY program (JMI Laboratories, Inc., North Liberty, IA).

## Competing interests

None declared.

## Ethical approval

Not required.

## References

[1] Dhand A, Sakoulas G. Reduced vancomycin susceptibility among clinical *Staphylococcus aureus* isolates ('the MIC creep'): implications for therapy. F1000 Med Rep 2012;4:4.
[2] Kehrmann J, Kaase M, Szabados F, Gatermann SG, Buer J, Rath PM, et al. Vancomycin MIC creep in MRSA blood culture isolates from Germany: a regional problem? Eur J Clin Microbiol Infect Dis 2011;30:677–83.
[3] Tomczak H, Szalek E, Blazejewska W, Myczko K, Horla A, Grzeskowiak E. The need to assay the real MIC when making the decision to eradicate *Staphylococcus aureus* with vancomycin. Postepy Hig Med Dosw (Online) 2013;67:921–5.
[4] Rossatto FC, Proenca LA, Becker AP, Silveira AC, Caierao J, D'Azevedo PA. Evaluation of methods in detecting vancomycin MIC among MRSA isolates and the changes in accuracy related to different MIC values. Rev Inst Med Trop Sao Paulo 2014;56:469–72.
[5] Kalil AC, Van Schooneveld TC, Fey PD, Rupp ME. Association between vancomycin minimum inhibitory concentration and mortality among patients with *Staphylococcus aureus* bloodstream infections: a systematic review and meta-analysis. JAMA 2014;312:1552–64.
[6] van Hal SJ, Barbagiannakos T, Jones M, Wehrhahn MC, Mercer J, Chen D, et al. Methicillin-resistant *Staphylococcus aureus* vancomycin susceptibility testing: methodology correlations, temporal trends and clonal patterns. J Antimicrob Chemother 2011;66:2284–7.
[7] Ligozzi M, Bernini C, Bonora MG, De Fatima M, Zuliani J, Fontana R. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant Gram-positive cocci. J Clin Microbiol 2002;40:1681–6.
[8] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. Document M07-A9. ninth edition Wayne, PA: CLSI; 2012.
[9] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. Document M100-S24. Wayne, PA: CLSI; 2014.
[10] Reynolds R, Hope R, Warner M, MacGowan AP, Livermore DM, Ellington MJ. Lack of upward creep of glycopeptide MICs for methicillin-resistant *Staphylococcus aureus* (MRSA) isolated in the UK and Ireland 2001–07. J Antimicrob Chemother 2012;67:2912–8.
[11] Jones RN. Microbiological features of vancomycin in the 21 st century: minimum inhibitory concentration creep, bactericidal/static activity, and applied breakpoints to predict clinical outcomes or detect resistant strains. Clin Infect Dis 2006;1(Suppl. 1):S13–24.
[12] Sader HS, Fey PD, Limaye AP, Madinger N, Pankey G, Rahal J, et al. Evaluation of vancomycin and daptomycin potency trends (MIC creep) against methicillin-resistant *Staphylococcus aureus* isolates collected in nine U.S. medical centers from 2002 to 2006. Antimicrob Agents Chemother 2009;53:4127–32.
[13] Robert J, Bismuth R, Jarlier V. Decreased susceptibility to glycopeptides in methicillin-resistant *Staphylococcus aureus*: a 20 year study in a large French teaching hospital, 1983–2002. J Antimicrob Chemother 2006;57:506–10.
[14] Wang G, Hindler JF, Ward KW, Bruckner DA. Increased vancomycin MICs for *Staphylococcus aureus* clinical isolates from a university hospital during a 5-year period. J Clin Microbiol 2006;44:3883–6.
[15] Steinkraus G, White R, Friedrich L. Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001–05. J Antimicrob Chemother 2007;60:788–94.
[16] Ho PL, Lo PY, Chow KH, Lau EH, Lai EL, Cheng VC, et al. Vancomycin MIC creep in MRSA isolates from 1997 to 2008 in a healthcare region in Hong Kong. J Infect 2010;60:140–5.
[17] Alos JI, Garcia-Canas A, Garcia-Hierro P, Rodriguez-Salvanes F. Vancomycin MICs did not creep in *Staphylococcus aureus* isolates from 2002 to 2006 in a setting with low vancomycin usage. J Antimicrob Chemother 2008;62:773–5.
[18] Musta AC, Riederer K, Shemes S, Chase P, Jose J, Johnson LB, et al. Vancomycin MIC plus heteroresistance and outcome of methicillin-resistant *Staphylococcus aureus* bacteremia: trends over 11 years. J Clin Microbiol 2009;47:1640–4.
[19] Gemmell CG, Edwards DI, Fraise AP, Gould FK, Ridgway GL, Warren RE. Guidelines for the prophylaxis and treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the UK. J Antimicrob Chemother 2006;57:589–608.
[20] Silvestre J, Póvoa P, Gonçalves E, Martins M. Is vancomycin MIC creep a worldwide phenomenon? Assessment of *S. aureus* vancomycin MIC in a tertiary university hospital. BMC Res Notes 2013;6:65.

# Part III. Discussion and conclusions

Regardless the big number of antistaphylococcal antibiotics that are available, the *S.aureus* ability to adapt makes it one of the principal causes of morbidity or even mortality (2) This microorganism is responsible both for nosocomial and community infections (13). Vancomycin is the "gold standard" for treatment of invasive MRSA infections (4). The work developed during the development of this thesis underline two points that can influence the treatment response in case of *S.aureus* infections: the possible presence of *mec*C gene and vancomycin MIC increase.

In 2011, *mec*C gene was found in MRSA isolates from humans and animals (9). This gene shares 69% nucleotide homology with *mec*A (9). This discovery created theories about the origin, epidemiology and impact of these isolates and the prevalence of *mec*C gene became an important public health issue. The study entitled "Methicillin-resistant *Staphylococcus aureus* carrying the new *mec*C gene—a meta-analysis" aimed to apply meta-analysis techniques to perform an improved evaluation of the available data on overall prevalence of *mec*CMRSA in humans and animals. With this study we found an estimated prevalence in the human subgroup of 0.004 (95% [CI] = 0.002–0.007), an estimated prevalence in the animal subgroup of 0.10 (95% [CI] = 0.033–0.174) and the overall estimated prevalence of 0.009 (95% [CI] = 0.005–0.013)(92). Several studies demonstrated that *mec*CMRSA is frequent in dairy cattle, suggesting that cows might provide a reservoir of infection (93), in fact, our results showed higher prevalence in animals subgroup when compared to the human subgroup and with dairy cattle as the most frequent subgroup with this infection. In humans, *mec*C gene has been predominantly described in skin and soft tissue infections, including fatal bacteremia (94). *mec*CMRSA was also found in urban wastewater, indicating that *mec*C gene can expand to new areas (95). Even though the origin of *mec*CMRSA is unclear, there is proof that animal contact poses a zoonotic risk and that *mec*CMRSA can be transmitted between different species (96).

The importance of monitor the presence of *mec*C in *S.aureus* isolates in both, humans and animals, lead to an update to the results of our previous published

meta-analysis and a new research was performed to include recent reports on *mec*C prevalence. This update, replicates literature review method reported but for articles published between April 2015 and August 2017. Ten new studies were selected based on the same inclusion and exclusion criteria (66, 97-105). These studies were added to the studies already included in the first meta-analysis. The estimated prevalence in the human subgroup was now 0,004 (95% [CI] = 0.002–0.007), the estimated prevalence in the animal subgroup 0,118 (95% [CI] = 0.063–0.179) and the overall prevalence 0.018 (95% [CI] = 0.012–0.024). The overall prevalence increased from 0.009 (95% [CI] = 0.005–0.013) to 0,018 (95% [CI] = 0.012–0.024). Although the estimated prevalence has doubled, the updated results did not show significant differences, since the 95% CI overlaps the previous one.

These updated results showed a slightly increased in two years and it is expect that a gradual increase over time will occur, namely in the animal subgroup, increasing the zoonotic risk and highlighting the importance of monitoring *S.aureus* strains carrying *mecC*. This updated literature search also noticed that *mec*C was described in three new countries, Italy (66), Australia (102) and Turkey (99) increasing its globalization. Gomez, *et al* (97) described *mec*CMRSA in red deer in Southern Spain (Cádiz) and it is known that these animals could potentially transmit this gene to other animals or even to humans. This geographic proximity to Portugal alerts to the fact that prevalence in Portugal can changed quickly. This possibility is sufficient to place Portuguese clinical laboratories in alert to this problem, because molecular techniques, such as PCR to detect MRSA or simpler techniques like slide agglutination do not detect this microorganism. With the use of these methodologies, for detection or confirmation of MRSA, we can obtained false-negative results, and *S.aureus* will be erroneously diagnosed as methicillin susceptible, potentially leading to wrong treatment decisions, increased delayed response, prolonged hospital stay and overall increased cost of hospitalization. In laboratories that perform antimicrobial susceptibility tests, *mec*CMRSA will likely be identified correctly as MRSA but when using automated VITEK2 system, *mec*CMRSA produce a distinctive antibiotic susceptibility profile when compared to *mec*A MRSA. This problem lead researchers to study *mec*CMRSA isolates on automated VITEK2

system and concluding that *mec*CMRSA was oxacillin susceptible and cefoxitin resistant (65). With this simple method used in clinical laboratories it is possible to suspect of the *mec*CMRSA and selecting the isolate for deeper analysis, namely, molecular techniques with primers able to amplify both *mec*A and *mec*C or only *mec*C gene. This strategy will also allow to locally monitoring the changes in *mec*CMRSA distribution and prevalence over time. Considering that contact with animals poses a zoonotic risk of infection with this gene and its report in neighbouring Spain and France both in animals and humans, advice to monitor its presence in Portuguese hospitals. The importance of locally monitor the changes in prevalence of *mec*CMRSA lead us to perform a study on rapid screening of MRSA carrying the *mec*C gene at the Centro Hospitalar Baixo Vouga was performed during research period (results submitted for peer review in international journal) with the aim of evaluate the presence of suspected *mec*CMRSA isolates, in Aveiro, Portugal, using a combination of two methods: the slide agglutination test and VITEK2 profile. Our sample included a retrospective analysis of oxacillin and cefoxitin obtained with automated system among 835 *S. aureus*, between January 2014 and December 2016. To test the inclusion of the combination of two methodologies in the day-to-day activities of the clinical laboratory a sample of 84 MRSA strains collected from Medicine department during 2014 were retested for slide agglutination test. To our knowledge *mec*CMRSA was not yet reported in Portugal and, in accordance, the results of our study do not suggest the presence of *mec*CMRSA in the studied sample but highlight the possibility of all clinical laboratories, to evaluate the presence of this microorganism and monitor changes in its prevalence over time, in a cheap, fast and simple away using standard methods, even for countries where *mec*CMRSA was not yet detected.

Monitoring the epidemiology of this emerging form of MRSA through persistent evaluation of *mec*C prevalence in humans and animals is fundamental to stimulate the development of procedures that allow the correct diagnostic of *mec*CMRSA. This effort will contribute to adequate treatment decisions when managing MRSA infection and reduced the overall increased cost of hospitalization if *mecC*MRSA arrives to Portugal.

There are differences described by Kim *et al* in biochemistry between *mec*A and *mec*C encoded PBP2a (63). The therapeutic implications of *mec*C have not been reported, but this variant PBP has a higher affinity for penicillins than for cephalosporins, and *in vitro*, *mec*C isolates are susceptible to the addition of clavulanate to penicillin (19, 106). This treatment may be a choice in future as an alternative to the "gold standard" vancomycin. This is particularly important given the increasing challenges posed by multidrug resistance and offers a hypothesis for confronting an emerging, resistant bacterial pathogen with an old antibiotic (106).

As mention *S.aureus* is a leading cause of nosocomial infections and treatment response could be influenced by increase in vancomycin MIC values. Previous studies have shown an association between high vancomycin MIC and treatment failure or mortality (107-110) other studies shown either an inverse association (111) or a lack of association (86, 112). The results are inconsistent since they do not show a close relationship between the MIC value and the outcome (113). Interestingly, some studies suggest that MRSA strains with elevated vancomycin MICs may be less virulent than strains with lower MICs (110, 113). Alterations in the cell wall structure and the low virulence of MRSA isolates with high vancomycin MIC could be related (110). In some studies it was suggested that the thickness of the cell wall in isolates with high vancomycin MICs covers the teichoic and lipoteichoic acids, inhibiting the immune activation and, subsequently, the development of shock. Additionally, MRSA isolates with a high MIC have been related with a slow growth rate and a loss of function of the accessory gene regulator operon (*agr*) that controls several virulence factors (114). Thus, there seems to be a relationship between pathogenicity and resistance to vancomycin. Loss of function of the accessory gene regulator operon might confer a survival advantage to MRSA under vancomycin pressure, mainly in isolates with the accessory gene regulator group II genotype (110, 115).

In recent years, it has been described a phenomenon called MIC creep, which describes the increase of the minimum inhibitory concentration of vancomycin in the susceptibility range. This issue started to be studied in various hospitals, mainly in Europe, to identify the best methods to evaluate and the variables that

affect it. In Portugal, the investigation of this phenomenon is still residual, only a study with data from Lisbon region between January 2007 and December 2009 was published and it reports data not compatible with the presence of MIC creep during the studied period (116).

There are researches with contradictory results when studying vancomycin MIC creep with MRSA. Some studies report MIC creep in some regions and large multicenter surveillance studies do not relate similar findings over time (116-118). Our research aimed to compare the population of Centro Hospitalar Baixo Vouga, Aveiro, with results already published at national and international level and produce information that will help monitoring this phenomenon. The study "Evaluation of vancomycin MIC creep in *Staphylococcus aureus* infections – a meta-analysis" assessed the global evidence of vancomycin MIC creep recurring to meta-analysis method. The sample included single and large multicenter reports published in Pubmed database. The results showed that mean values of vancomycin MICs, of all MRSA isolates reported, were 1.19 mg/L and 1.20 mg/L determined by Etest and BMD method, respectively.

When considering pooled mean of vancomycin MIC over time, it was showed that studies published before the year 2000 exhibited the highest vancomycin MICs and after 2007 the vancomycin MICs showed a slight decrease. The results were similar for the two selected MIC methods, BMD and Etest. Correlations between MIC testing methods effect and time strata were studied and no evidence of MIC creep were observed. When analyzing MRSA isolates that reported vancomycin MIC ≥ 2 mg/L over time strata, both BMD and Etest methods show a decrease in vancomycin MIC, strengthening the observation of no evidence of MIC creep.

It is important to understand that data from multiple centres can obscure trends that may exist within a given institution or country, as a result of differences in patient populations and drug usage patterns. However, the negative values of Spearman´s correlation obtained in our meta-analysis support no evidence of vancomycin MIC creep.

Analyse vancomycin MIC creep linked to a regional problem and evaluate local susceptibility profiles is important to created data that will help in local MRSA infection clinical management. Kehrmann *et al*., assessed the vancomycin MIC

distribution for MRSA blood culture isolates during 6 years in Germany. MRSA isolates were collected at several hospitals in two German cities and MIC creep phenomenon was detected only in one city, suggesting that this phenomenon could be a regional problem and recommending that all hospitals should monitor their local status (118). To evaluate this hypothesis in the study "Evaluation of vancomycin MIC creep in *Staphylococcus aureus* infections" we aimed to assess vancomycin MIC distribution for *S.aureus* infections, over a period of four years, in Centro Hospitalar Baixo Vouga, Aveiro, Portugal and evaluate differences in vancomycin MIC through the application of different susceptibility testing methods. The results showed significant differences of vancomycin MIC by difference methods but do not suggest the presence of vancomycin MIC creep during the study period. These results showed that vancomycin MICs from the automated method were also significantly lower than those found by the BMD analysis. It is important to highlight that real MIC assayed by means of Etest or BMD is more useful than that provided by the automated method (5). These findings are consistent with the results obtained in the study of Tomczak H. *et al* that compared MIC obtained with the automated method and with the Etest method for *S.aureus* strains isolated from clinical materials and concluded that results obtained with automated method were lower (5).

These results and knowing that the type of microbiological susceptibility used, type of *S.aureus* strain examined or type of patient population evaluated, may influence MIC creep phenomenon, we suggest that all hospitals should monitor their local status of vancomycin MICs to evaluate the presence of trends and ensure the effectiveness of therapy with vancomycin, preferably with Etest or BMD methods.

At Centro Hospitalar Baixo Vouga, Aveiro, we can conclude that treatment decisions and vancomycin MIC value are being applied in a way that, for the period studied, did not create a MIC creep phenomenon, suggesting that management MRSA infection with vancomycin is on the right path. The importance of vancomycin MIC in the prognosis of MRSA infection is widely recognized, as there is clear evidence of loss of efficacy of vancomycin with MIC greater than or equal to 1mg/ml, justifying the use of other drugs, namely

linezolid, daptomycin and tigecycline, especially when the infection is located in organs where the penetration of the antibiotic is more complex.

The correct antibiotic therapy used in a early stage is essential in treatment of patients. But, in the majority of cases, the initial decision about the antibiotic therapy is made empirically, while awaiting the microbiology results. Current evidence is insufficient to support empiric use of alternative agents such linezolid or daptomycin, for reasons like: broader use of these alternative agents will likely lead to increasing resistance to them; frank vancomycin resistance remains rare and only a fraction of the patients with suspected MRSA showed a vancomycin MIC high but in the susceptible range. Nevertheless, is extremely relevant that our institution continues to monitor the vancomycin MICs to evaluate the possible presence of trends in MIC over time, allowing antibiotic therapy choices as accurate as possible.

Either with the evaluation of the MIC values of vancomycin or with research of the *mec*C gene in *S. aureus*, the purpose of this work is based on the need to better understand the resistance of nosocomial infections to antibiotics and to alert clinicians to the possibility of resistance to vancomycin scenario and the microbiology laboratories for the risk of new strains carrying the resistance genes.

In conclusion, the discovery of the *mec*C gene can represent a public health threat and although this gene was not detected in Portugal it is important that all laboratories should revise the diagnostic protocols for this new gene. This gene was mainly found in livestock-associated MRSA and its prevalence is clearly increasing since it was reported. The therapeutic implications of *mec*C have not been reported, but there are speculations about the treatment of this new variant with old antibiotics that could be an alternative to the gold standard vancomycin.

On the other hand, no evidence of vancomycin MIC creep was found, either in the meta-analysis or in our institution. Even so, these results must be considered when interpreting vancomycin susceptibility and during discussions about alternative antistaphylococcal agents for patients because the present strategy cloud work today but became ineffective rapidly if MIC creep emerge inside the institution.

# Part IV. Future perspectives

Monitor the presence of *mec*CMRSA in regions with high prevalence of MRSA, should be consider and Portugal, a country with high MRSA prevalence, is a good example for that. The zoonotic risk of *mec*CMRSA infections motivates the future evaluation of its prevalence between different livestock species. It is also important to understand, for future studies, their role in animal diseases and its zoonotic transmission mechanism. The link of *mec*C to the resistance mechanism and to the susceptibility of the strain on antibiotic treatment must also be evaluated since it is foreseen that it will probably contribute for the future difficulties that society will need to transpose when managing bacterial resistance. In this spectrum, the implementation of systematic review of susceptibility profiles of bacteria to antibiotics must became permanent and made locally to better understand the differences between regions and populations. The evaluation of vancomycin MIC trend in our hospital demonstrates utility to the health professional that work with this antibiotic. Reproduce this approach systematically for vancomycin in other Portuguese hospitals and also to other susceptibility situation is without doubts a road to take in future research.

# Part V. References

1. McGuinness WA, Malachowa N, DeLeo FR. Vancomycin Resistance in Staphylococcus aureus . The Yale Journal of Biology and Medicine. 2017;90(2):269-81.

2. Dennis Kasper AF. Staphylococcal Infections. In: Hill MG, editor. Harrison´s Infections Diseases2010. p. 386-99.

3. Levine DP. Vancomycin: a history. Clin Infect Dis. 2006;42 Suppl 1:S5-12.

4. Rubinstein E, Keynan Y. Vancomycin revisited - 60 years later. Front Public Health. 2014;2(217).

5. Tomczak H, Szalek E, Blazejewska W, Myczko K, Horla A, Grzeskowiak E. The need to assay the real MIC when making the decision to eradicate Staphylococcus aureus with vancomycin. Postepy Hig Med Dosw (Online). 2013;67:921-5.

6. Dhand A, Sakoulas G. Reduced vancomycin susceptibility among clinical Staphylococcus aureus isolates ('the MIC Creep'): implications for therapy. F1000 Med Rep. 2012;4(4):1.

7. Rossatto FC, Proenca LA, Becker AP, Silveira AC, Caierao J, D'Azevedo PA. Evaluation of methods in detecting vancomycin MIC among MRSA isolates and the changes in accuracy related to different MIC values. Rev Inst Med Trop Sao Paulo. 2014;56(6):469-72.

8. Kalil AC, Van Schooneveld TC, Fey PD, Rupp ME. Association between vancomycin minimum inhibitory concentration and mortality among patients with Staphylococcus aureus bloodstream infections: a systematic review and meta-analysis. JAMA. 2014;312(15):1552-64.

9. Ito T, Hiramatsu K, Tomasz A, de Lencastre H, Perreten V, Holden MT, et al. Guidelines for reporting novel mecA gene homologues. Antimicrob Agents Chemother. 2012;56(10):4997-9.

10. Paterson GK, Harrison EM, Holmes MA. The emergence of mecC methicillin-resistant Staphylococcus aureus. Trends in Microbiology. 2014;22(1):42-7.

11. Wilson LG. The early recognition of streptococci as causes of disease. Med Hist. 1987;31(4):403-14.

12. Corey GR. Staphylococcus aureus Bloodstream Infections: Definitions and Treatment. Clinical Infectious Diseases. 2009;48(Supplement 4):S254-S9.

13. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603-61.

14. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis. 2005;5(12):751-62.

15. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev. 1997;10(3):505-20.

16. Emori TG, Gaynes RP. An overview of nosocomial infections, including the role of the microbiology laboratory. Clin Microbiol Rev. 1993;6(4):428-42.

17. Archer GL. Staphylococcus aureus: a well-armed pathogen. Clin Infect Dis. 1998;26(5):1179-81.

18. Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant Staphylococcus aureus infection. Clin Infect Dis. 2008;1(46):350-9.

19. Moreilon Y-AQaP. Staphylococcus aureus (Including Staphylococcal Toxic shock Syndrome). Principles and Practice of Infectious Diseases. Eighth Edition ed2015.

20. Novick RP. Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol. 2003;48(6):1429-49.

21. Novick RP, Ross HF, Projan SJ, Kornblum J, Kreiswirth B, Moghazeh S. Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. EMBO J. 1993;12(10):3967-75.

22. Cheung AL, Bayer AS, Zhang G, Gresham H, Xiong YQ. Regulation of virulence determinants in vitro and in vivo in Staphylococcus aureus. FEMS Immunol Med Microbiol. 2004;40(1):1-9.

23. Peacock SJ, Moore CE, Justice A, Kantzanou M, Story L, Mackie K, et al. Virulent combinations of adhesin and toxin genes in natural populations of Staphylococcus aureus. Infect Immun. 2002;70(9):4987-96.

24. Foster TJ, Hook M. Surface protein adhesins of Staphylococcus aureus. Trends Microbiol. 1998;6(12):484-8.

25. Weidenmaier C, Kokai-Kun JF, Kristian SA, Chanturiya T, Kalbacher H, Gross M, et al. Role of teichoic acids in Staphylococcus aureus nasal colonization, a major risk factor in nosocomial infections. Nat Med. 2004;10(3):243-5.

26. von Eiff C, Friedrich AW, Peters G, Becker K. Prevalence of genes encoding for members of the staphylococcal leukotoxin family among clinical isolates of Staphylococcus aureus. Diagn Microbiol Infect Dis. 2004;49(3):157-62.

27. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 2003;9(8):978-84.

28. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, et al. Methicillin-resistant S. aureus infections among patients in the emergency department. N Engl J Med. 2006;355(7):666-74.

29. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, et al. Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis. 2001;32 Suppl 2:S114-32.

30. Rodriguez-Creixems M, Alcala L, Munoz P, Cercenado E, Vicente T, Bouza E. Bloodstream infections: evolution and trends in the microbiology workload, incidence, and etiology, 1985-2006. Medicine (Baltimore). 2008;87(4):234-49.

31. Cosgrove SE, Fowler VG, Jr. Management of methicillin-resistant Staphylococcus aureus bacteremia. Clin Infect Dis. 2008;46 Suppl 5:S386-93.

32. Lowy FD. Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest. 2003;111(9):1265-73.

33. Peacock SJ, Paterson GK. Mechanisms of Methicillin Resistance in Staphylococcus aureus. Annu Rev Biochem. 2015;84:577-601.

34. Moellering RC, Jr. MRSA: the first half century. J Antimicrob Chemother. 2012;67(1):4-11.

35. Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol. 2009;7(9):629-41.

36. Rodvold KA, McConeghy KW. Methicillin-resistant Staphylococcus aureus therapy: past, present, and future. Clin Infect Dis. 2014;58(1).

37. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children: executive summary. Clin Infect Dis. 2011;52(3):285-92.

38. Hanssen AM, Ericson Sollid JU. SCCmec in staphylococci: genes on the move. FEMS Immunol Med Microbiol. 2006;46(1):8-20.

39. Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K. Insights on antibiotic resistance of Staphylococcus aureus from its whole genome: genomic island SCC. Drug Resist Updat. 2003;6(1):41-52.

40. Hiramatsu K, Ito T, Tsubakishita S, Sasaki T, Takeuchi F, Morimoto Y, et al. Genomic Basis for Methicillin Resistance in Staphylococcus aureus. Infect Chemother. 2013;45(2):117-36.

41. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. Antimicrob Agents Chemother. 2000;44(6):1549-55.

42. elements IWGotSCC. International Working Group on the Staphylococcal Cassette Chromosome elements 2017 [27 September 2016]. The aim of website is to provide up to date Information on the SCCmec and SCC elements]. Available from: http://www.sccmec.org/.

43. Liu J, Chen D, Peters BM, Li L, Li B, Xu Z, et al. Staphylococcal chromosomal cassettes mec (SCCmec): A mobile genetic element in methicillin-resistant Staphylococcus aureus. Microb Pathog. 2016;101:56-67.

44. Katayama Y, Ito T, Hiramatsu K. Genetic organization of the chromosome region surrounding mecA in clinical staphylococcal strains: role of IS431-mediated mecI deletion in expression of resistance in mecA-carrying, low-level methicillin-resistant Staphylococcus haemolyticus. Antimicrob Agents Chemother. 2001;45(7):1955-63.

45. Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother. 2009;53(12):4961-7.

46. Shore AC, Coleman DC. Staphylococcal cassette chromosome mec: recent advances and new insights. Int J Med Microbiol. 2013;303(6-7):350-9.

47. Giesbrecht P, Kersten T, Maidhof H, Wecke J. Staphylococcal cell wall: morphogenesis and fatal variations in the presence of penicillin. Microbiol Mol Biol Rev. 1998;62(4):1371-414.

48. Fuda C, Suvorov M, Vakulenko SB, Mobashery S. The basis for resistance to beta-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant Staphylococcus aureus. J Biol Chem. 2004;279(39):40802-6.

49. Berger-Bachi B, Rohrer S. Factors influencing methicillin resistance in staphylococci. Arch Microbiol. 2002;178(3):165-71.

50. Deurenberg RH, Stobberingh EE. The evolution of Staphylococcus aureus. Infect Genet Evol. 2008;8(6):747-63.

51. Rolo J, Miragaia M, Turlej-Rogacka A, Empel J, Bouchami O, Faria NA, et al. High genetic diversity among community-associated Staphylococcus aureus in Europe: results from a multicenter study. PLoS One. 2012;7(4):27.

52. Bronzwaer SL, Goettsch W, Olsson-Liljequist B, Wale MC, Vatopoulos AC, Sprenger MJ. European Antimicrobial Resistance Surveillance System (EARSS): objectives and organisation. Euro Surveill. 1999;4(4):41-4.

53. Tiemersma EW, Bronzwaer SL, Lyytikainen O, Degener JE, Schrijnemakers P, Bruinsma N, et al. Methicillin-resistant Staphylococcus aureus in Europe, 1999-2002. Emerg Infect Dis. 2004;10(9):1627-34.

54. (ECDC) ECfDPaC. European Centre for Disease Prevention and Control 2017 [cited 2017 August 2017 ]. Surveillance of microorganisms and disease]. Available from: http://ecdc.europa.eu/en/Pages/home.aspx.

55. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Meticillin-resistant Staphylococcus aureus (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents. 2012;39(4):273-82.

56. McDonald LC. Trends in antimicrobial resistance in health care-associated pathogens and effect on treatment. Clin Infect Dis. 2006;42 Suppl 2:S65-71.

57. Feil EJ, Cooper JE, Grundmann H, Robinson DA, Enright MC, Berendt T, et al. How clonal is Staphylococcus aureus? J Bacteriol. 2003;185(11):3307-16.

58. Sung JY, Lee J, Choi EH, Lee HJ. Changes in molecular epidemiology of community-associated and health care-associated methicillin-resistant Staphylococcus aureus in Korean children. Diagn Microbiol Infect Dis. 2012;74(1):28-33.

59. Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, et al. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA). J Antimicrob Chemother. 2005;56(6):1000-18.

60. Diseases. ESoMaI. Breakpoint tables for interpretation of MICs and zone. Version 5.0. 2015 [cited 2017 August 2017]. Breakpoint tables for interpretation of MICs and zone]. Available from: http://www.eucast.org/clinical_breakpoints/.

61. Paterson GK, Morgan FJ, Harrison EM, Cartwright EJ, Torok ME, Zadoks RN, et al. Prevalence and characterization of human mecC methicillin-resistant Staphylococcus aureus isolates in England. J Antimicrob Chemother. 2013;27:907-10.

62. Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, et al. Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis. 2011;11(8):595-603.

63. Kim C, Milheirico C, Gardete S, Holmes MA, Holden MT, de Lencastre H, et al. Properties of a novel PBP2A protein homolog from Staphylococcus aureus strain LGA251 and its contribution to the beta-lactam-resistant phenotype. J Biol Chem. 2012;287(44):36854-63.

64. Skov R, Larsen AR, Kearns A, Holmes M, Teale C, Edwards G, et al. Phenotypic detection of mecC-MRSA: cefoxitin is more reliable than oxacillin. J Antimicrob Chemother. 2014;69(1):133-5.

65. Cartwright EJ, Paterson GK, Raven KE, Harrison EM, Gouliouris T, Kearns A, et al. Use of Vitek 2 antimicrobial susceptibility profile to identify mecC in methicillin-resistant Staphylococcus aureus. J Clin Microbiol. 2013;51(8):2732-4.

66. Giacinti G, Carfora V, Caprioli A, Sagrafoli D, Marri N, Giangolini G, et al. Prevalence and characterization of methicillin-resistant Staphylococcus aureus carrying mecA or mecC and methicillin-susceptible Staphylococcus aureus in dairy sheep farms in central Italy. J Dairy Sci. 2017.

67. Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in Staphylococcus aureus, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev. 2010;23(1):99-139.

68. Reynolds PE. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. Eur J Clin Microbiol Infect Dis. 1989;8(11):943-50.

69. Courvalin P. Vancomycin resistance in gram-positive cocci. Clin Infect Dis. 2006;42 Suppl 1:S25-34.

70. Rybak M, Lomaestro B, Rotschafer JC, Moellering R, Jr., Craig W, Billeter M, et al. Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. Am J Health Syst Pharm. 2009;66(1):82-98.

71. Kollef MH. Limitations of vancomycin in the management of resistant staphylococcal infections. Clin Infect Dis. 2007;45 Suppl 3:S191-5.

72. Forouzesh A, Moise PA, Sakoulas G. Vancomycin ototoxicity: a reevaluation in an era of increasing doses. Antimicrob Agents Chemother. 2009;53(2):483-6.

73. Avent ML, Vaska VL, Rogers BA, Cheng AC, van Hal SJ, Holmes NE, et al. Vancomycin therapeutics and monitoring: a contemporary approach. Intern Med J. 2013;43(2):110-9.

74. Bailie GR, Neal D. Vancomycin ototoxicity and nephrotoxicity. A review. Med Toxicol Adverse Drug Exp. 1988;3(5):376-86.

75. Noble WC, Virani Z, Cree RG. Co-transfer of vancomycin and other resistance genes from Enterococcus faecalis NCTC 12201 to Staphylococcus aureus. FEMS Microbiol Lett. 1992;72(2):195-8.

76. Vancomycin-resistant Staphylococcus aureus--New York, 2004. MMWR Morb Mortal Wkly Rep. 2004;53(15):322-3.

77. Vancomycin-resistant Staphylococcus aureus--Pennsylvania, 2002. MMWR Morb Mortal Wkly Rep. 2002;51(40):902.

78. Staphylococcus aureus resistant to vancomycin--United States, 2002. MMWR Morb Mortal Wkly Rep. 2002;51(26):565-7.

79. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother. 1997;40(1):135-6.

80. Murray BE. Vancomycin-resistant enterococcal infections. N Engl J Med. 2000;342(10):710-21.

81. Chang S, Sievert DM, Hageman JC, Boulton ML, Tenover FC, Downes FP, et al. Infection with vancomycin-resistant Staphylococcus aureus containing the vanA resistance gene. N Engl J Med. 2003;348(14):1342-7.

82. Brink AJ. Does resistance in severe infections caused by methicillin-resistant Staphylococcus aureus give you the 'creeps'? Curr Opin Crit Care. 2012;18(5):451-9.

83. Clinical and Laboratory Standards Institute C. Methods for Dilution Antimicrobial Susceptibility Tests f or Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. 2012;29.

84. Himani, Agrawal C, Madan M, Pandey A, Thakuria B. Methicillin Resistant Staphylococcus aureus: Inconsistencies in Vancomycin Susceptibility Testing Methods, Limitations and Advantages of each Method. Journal of Clinical and Diagnostic Research : JCDR. 2015;9(10):DC01-DC4.

85. van Hal SJ, Lodise TP, Paterson DL. The clinical significance of vancomycin minimum inhibitory concentration in Staphylococcus aureus infections: a systematic review and meta-analysis. Clin Infect Dis. 2012;54(6):755-71.

86. Musta AC, Riederer K, Shemes S, Chase P, Jose J, Johnson LB, et al. Vancomycin MIC plus heteroresistance and outcome of methicillin-resistant Staphylococcus aureus bacteremia: trends over 11 years. J Clin Microbiol. 2009;47(6):1640-4.

87. Steinkraus G, White R, Friedrich L. Vancomycin MIC creep in non-vancomycin-intermediate Staphylococcus aureus (VISA), vancomycin-susceptible clinical methicillin-resistant S. aureus (MRSA) blood isolates from 2001-05. J Antimicrob Chemother. 2007;60(4):788-94.

88. (ISO) TIOfS. Clinical laboratory testing and in vitro diagnostic test systems 2006. Available from: https://www.iso.org/obp/ui/ - iso:std:iso:20776:-1:ed-1:v1:en.

89. Jenkins SG, Schuetz AN. Current concepts in laboratory testing to guide antimicrobial therapy. Mayo Clin Proc. 2012;87(3):290-308.

90. Chaudhari CN, Tandel K, Grover N, Bhatt P, Sahni AK, Sen S, et al. In vitro vancomycin susceptibility amongst methicillin resistant Staphylococcus aureus. Med J Armed Forces India. 2014;70(3):215-9.

91. Ligozzi M, Bernini C, Bonora MG, De Fatima M, Zuliani J, Fontana R. Evaluation of the VITEK 2 system for identification and antimicrobial

susceptibility testing of medically relevant gram-positive cocci. J Clin Microbiol. 2002;40(5):1681-6.

92. Diaz R, Ramalheira E, Afreixo V, Gago B. Methicillin-resistant Staphylococcus aureus carrying the new mecC gene-a meta-analysis. Diagn Microbiol Infect Dis. 2016;84(2):135-40.

93. Gomez P, Gonzalez-Barrio D, Benito D, Garcia JT, Vinuela J, Zarazaga M, et al. Detection of methicillin-resistant Staphylococcus aureus (MRSA) carrying the mecC gene in wild small mammals in Spain. J Antimicrob Chemother. 2014;69(8):2061-4.

94. Garcia-Garrote F, Cercenado E, Marin M, Bal M, Trincado P, Corredoira J, et al. Methicillin-resistant Staphylococcus aureus carrying the mecC gene: emergence in Spain and report of a fatal case of bacteraemia. J Antimicrob Chemother. 2014;69(1):45-50.

95. Concepcion Porrero M, Harrison EM, Fernandez-Garayzabal JF, Paterson GK, Diez-Guerrier A, Holmes MA, et al. Detection of mecC-Methicillin-resistant Staphylococcus aureus isolates in river water: a potential role for water in the environmental dissemination. Environ Microbiol Rep. 2014;6(6):705-8.

96. Paterson GK, Morgan FJ, Harrison EM, Cartwright EJ, Torok ME, Zadoks RN, et al. Prevalence and characterization of human mecC methicillin-resistant Staphylococcus aureus isolates in England. J Antimicrob Chemother. 2014;69(4):907-10.

97. Gomez P, Lozano C, Gonzalez-Barrio D, Zarazaga M, Ruiz-Fons F, Torres C. High prevalence of methicillin-resistant Staphylococcus aureus (MRSA) carrying the mecC gene in a semi-extensive red deer (Cervus elaphus hispanicus) farm in Southern Spain. Vet Microbiol. 2015;177(3-4):326-31.

98. Haenni M, Chatre P, Dupieux C, Metayer V, Maillard K, Bes M, et al. mecC-positive MRSA in horses. J Antimicrob Chemother. 2015;70(12):3401-2.

99. Kilic A, Dogan E, Kaya S, Baysallar M. [Investigation of the presence of mecC and Panton-Valentine leukocidin genes in Staphylococcus aureus strains isolated from clinical specimens during seven years period]. Mikrobiyol Bul. 2015;49(4):594-9.

100. Benito D, Gomez P, Aspiroz C, Zarazaga M, Lozano C, Torres C. Molecular characterization of Staphylococcus aureus isolated from humans related to a livestock farm in Spain, with detection of MRSA-CC130 carrying mecC gene: A zoonotic case? Enferm Infecc Microbiol Clin. 2016;34(5):280-5.

101. Gomez P, Lozano C, Camacho MC, Lima-Barbero JF, Hernandez JM, Zarazaga M, et al. Detection of MRSA ST3061-t843-mecC and ST398-t011-mecA in white stork nestlings exposed to human residues. J Antimicrob Chemother. 2016;71(1):53-7.

102. Worthing KA, Coombs GW, Pang S, Abraham S, Saputra S, Trott DJ, et al. Isolation of mecC MRSA in Australia. J Antimicrob Chemother. 2016;71(8):2348-9.

103. Angen O, Stegger M, Larsen J, Lilje B, Kaya H, Pedersen KS, et al. Report of mecC-carrying MRSA in domestic swine. J Antimicrob Chemother. 2017;72(1):60-3.

104. Bengtsson B, Persson L, Ekstrom K, Unnerstad HE, Uhlhorn H, Borjesson S. High occurrence of mecC-MRSA in wild hedgehogs (Erinaceus europaeus) in Sweden. Vet Microbiol. 2017;207:103-7.

105. Bortolami A, Verin R, Chantrey J, Corro M, Ashpole I, Lopez J, et al. Characterization of Livestock-Associated Methicillin-Resistant Staphylococcus aureus CC398 and mecC-positive CC130 from Zoo Animals in the United Kingdom. Microb Drug Resist. 2017.

106. Ba X, Harrison EM, Lovering AL, Gleadall N, Zadoks R, Parkhill J, et al. Old Drugs To Treat Resistant Bugs: Methicillin-Resistant Staphylococcus aureus Isolates with mecC Are Susceptible to a Combination of Penicillin and Clavulanic Acid. Antimicrob Agents Chemother. 2015;59(12):7396-404.

107. Haque NZ, Zuniga LC, Peyrani P, Reyes K, Lamerato L, Moore CL, et al. Relationship of vancomycin minimum inhibitory concentration to mortality in patients with methicillin-resistant Staphylococcus aureus hospital-acquired, ventilator-associated, or health-care-associated pneumonia. Chest. 2010;138(6):1356-62.

108. Lodise TP, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro BM, et al. Relationship between vancomycin MIC and failure among patients with methicillin-resistant Staphylococcus aureus bacteremia treated with vancomycin. Antimicrob Agents Chemother. 2008;52(9):3315-20.

109. Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering RC, Jr., Eliopoulos GM. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant Staphylococcus aureus bacteremia. J Clin Microbiol. 2004;42(6):2398-402.

110. Soriano A, Marco F, Martinez JA, Pisos E, Almela M, Dimova VP, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant Staphylococcus aureus bacteremia. Clin Infect Dis. 2008;46(2):193-200.

111. Price J, Atkinson S, Llewelyn M, Paul J. Paradoxical relationship between the clinical outcome of Staphylococcus aureus bacteremia and the minimum inhibitory concentration of vancomycin. Clin Infect Dis. 2009;48(7):997-8.

112. Beeston CJ, Gupta R, Chadwick PR, Young RJ. Methicillin-resistant Staphylococcus aureus bacteraemia and mortality in a teaching hospital. Eur J Clin Microbiol Infect Dis. 2009;28(6):585-90.

113. Clemens EC, Chan JD, Lynch JB, Dellit TH. Relationships between vancomycin minimum inhibitory concentration, dosing strategies, and outcomes in methicillin-resistant Staphylococcus aureus bacteremia. Diagn Microbiol Infect Dis. 2011;71(4):408-14.

114. Sakoulas G, Moellering RC, Jr., Eliopoulos GM. Adaptation of methicillin-resistant Staphylococcus aureus in the face of vancomycin therapy. Clin Infect Dis. 2006;42 Suppl 1:S40-50.

115. Han JH, Mascitti KB, Edelstein PH, Bilker WB, Lautenbach E. Effect of reduced vancomycin susceptibility on clinical and economic outcomes in Staphylococcus aureus bacteremia. Antimicrob Agents Chemother. 2012;56(10):5164-70.

116. Joana S, Pedro P, Elsa G, Filomena M. Is vancomycin MIC creep a worldwide phenomenon? Assessment of S. aureus vancomycin MIC in a tertiary university hospital. BMC Res Notes. 2013;6(65).

117. Sader HS, Fey PD, Limaye AP, Madinger N, Pankey G, Rahal J, et al. Evaluation of vancomycin and daptomycin potency trends (MIC creep) against methicillin-resistant Staphylococcus aureus isolates collected in nine U.S. medical centers from 2002 to 2006. Antimicrob Agents Chemother. 2009;53(10):4127-32.

118. Kehrmann J, Kaase M, Szabados F, Gatermann SG, Buer J, Rath PM, et al. Vancomycin MIC creep in MRSA blood culture isolates from Germany: a regional problem? Eur J Clin Microbiol Infect Dis. 2011;30(5):677-83.

Raquel Diaz

raqueldiaz85@gmail.com / raqueldiaz@ua.pt

June 2018